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A STUDY OF THE STRUCTURE OF SUGAR BEETS IN RELATION TO SUGAR CONTENT AND TYPE[1]

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INTRODUCTION

In order to recognize a plant type and to learn its inherent possibilities it is necessary to delimit it sharply. This has been done for most cultivated plants, but beet fields often show even within a limited area plants of most diversified root and foliage characters. The system of mass selection used in beet-breeding work is largely responsible for this diversity. The commercial beets have been bred for performance, and whenever the performance of certain seed lots sank below a certain level new "families" were introduced. Perhaps the principal reason for not employing pure-line selection methods has been the common belief in the protandry of the beet flower. Recent investigations, however, have greatly weakened the foundation for this concept, for it is now an established fact that close fertilization in beet flowers is theoretically possible (*1*),[3] and it seems probable that failures in pure-line work may have been due to self-incompatibility or certain other factors; but that this condition may not necessarily be a stumbling block has been suggested by many workers (Munerati, Vilmorin, Nilsson), who vigorously combat the general commercial viewpoint that pure-line methods have no applicability in sugar-beet breeding work.

Most of the morphologically uniform beet selections in this country were the outcome of the indefatigable labors of W. W. Tracy, formerly at the sugar-plant breeding station of the United States Department of Agriculture at Fort Collins, Colo. Tracy's lines were originally selected out of ordinary commercial stock. All samples were first bred by isolating roots individually from two to four generations. After this number of generations of individual isolations of single roots, the samples were in many cases thrown into group isolations. The mother beets of the various strains were grown separately under good conditions of isolation in order to prevent cross pollination with other beets.

The work reported in this paper is an anatomical study of a number of Tracy's selections for the purpose of finding characters that would aid in determining the purity of a type, in delimiting it more sharply,

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[2] The writer is under obligation to W. W. Tracy and D. W. Koonce for growing the material used in this study, to S. F. Sherwood and Mrs. Ruth C. Starrett for the sugar analyses, and to Mrs. E. Artschwager for making the drawings.

[3] Reference is made by number (italic) to "Literature cited," p. 914.

and in testing on a larger scale than has hitherto been done the various theories of a possible connection between anatomical structure and sugar content.

Although the strains examined were far more uniform than ordinary commercial stock, they do not yet constitute pure lines, and many of the discrepancies in the anatomical characterization of the types are the expression of different line impurities.

MATERIALS AND METHODS

The material studied in 1927 consisted of 700 beets taken from 30 of Tracy's selections. A description of each selection is given in Table 1. The beets were harvested during the first week in October and were analyzed and examined directly in the field laboratory. The beets tested rather low because the season was in general unfavorable for high sugar production and the beets were harvested two weeks in advance of the regular digging. The unevenness in percentage of sucrose was partly the result of leaf-spot infection, from which some plants recovered earlier than others and were able to store a larger amount of sugar. In 1928 only 23 of the original 30 selections were available for study, but the material was grown in two places, at Fort Collins and Fort Lewis, Colo. One thousand beets were harvested the second week in October and were carefully packed and shipped to Washington, D. C., for further study.

The data were taken on cross sections through the neck region of the beet. This region appears to be the best suited for this study, and a number of earlier investigations have been based on it. But even here the outline is not always symmetrical, which makes it difficult to obtain correct ring counts and decreases the accuracy of the measurements. The total diameter of the beet is the mean of the short and long diameters wherever the circumference is not a circle. This applies also to the measurements of the first ring and the central core. The bundles of the vascular rings were counted in stained sections. The topped beet was placed with the neck region in a shallow dish containing Delafield's haematoxylin. The dish with the beet was then transferred to a bell jar, from which the air was subsequently exhausted. After a few minutes the beet was removed from the jar and thin slices were cut, usually with the aid of a slicer. These were examined on an opal glass plate with bottom illumination. The sections appeared white, except for the xylem of the bundles, which stood out a bright purple against the colorless background. The size of the interzonal parenchyma cells was determined by projecting a slide on a white screen and counting the cells of a given area.

The photographs of the beet cross sections are from fresh unstained material taken on panchromatic plates. The central cores were stained with phloroglucin and hydrochloric acid in order to obtain the greatest possible contrast between vascular tissue and parenchyma. The drawings of the vascular bundles are from photomicrographs taken directly on bromide paper. All other photomicrographs were taken on Wratten M plates with B–58 and E–22 filters used singly or in combination.

TABLE 1.—*Description of the 30 sugar-beet selections studied*

[Selections listed in numerical order]

Selection No.	Plant		Foliage		
	Type	Size	Number of leaves	Size	Spread of foliage
150-24	Uniform	Medium	Somewhat large	Medium	Semierect.
193R-25	do	Somewhat small	Somewhat small	Somewhat large	Erect.
554-24	Medium	do	Very large	Somewhat small	Spreading.
600s-25	Uniform	Somewhat large	Small	Medium	Do.
730-24	Medium	Large	do	Somewhat large	Semierect.
898-24	Uniform	Small	Large	do	Decidedly spreading.
992s-25	do	Large	Somewhat small	Medium	Semierect.
1161-24	do	Medium	Somewhat large	Somewhat large	Do.
1228s-25	do	Somewhat large	Small	Small	Spreading.
1293s-25	Unlike	Somewhat small	Medium	Medium	Somewhat spreading.
1427-24	Uniform	Somewhat large	Somewhat small	do	Semispreading.
1569-24	Very uniform	Small	Small	Small	Spreading.
1591-24	Uniform	Large	Somewhat large	Medium	Semierect.
1612-24	do	do	do	do	Somewhat spreading.
1818s-25	do	Medium	do	do	Semierect.
1845s-25	Medium	do	Medium	Small	Somehwat spreading.
2287-24	Uniform	Large	Small	Decidedly large	Erect.
2310s-25	do	Decidedly large	Medium	Medium	Do.
2346s-25	Unlike	Medium	Somewhat small	Somewhat large	Somewhat spreading.
2445s-25	Uniform	do	Large		Semierect.
3403-24	Unlike	do	do	Somewhat large	Do.
3956-24	Medium	Large	Somewhat small	Decidedly small	Very erect.
4545-24	Uniform	do	Small	Large	Erect.
4621-22	Unlike	do	Medium	Small	Somewhat spreading.
5656-24	Medium	Medium	Somewhat small	Large	Erect.
5942-24	Uniform	do	Somewhat large	Somewhat small	Semierect.
6077-24	do	Large	do	do	Somewhat spreading.
7794-24	Medium	Medium	Large	Medium	Semierect.
8146-24	Uniform	Somewhat large	Somewhat small	Large	Spreading.
8753-24	do	Medium	Large	Medium	Semierect.

Selection No.	Root				
	Size	Shape	Length	Fullness	Taproot
150-24	Medium	Top	Slender, short	Semidepressed	Large.
193R-25	Somewhat small	Parsnip-top	do	Depressed	Decidedly large.
554-24	Small	do	Slender	do	Very large.
600s-25	Decidedly large	Mangel-top	Slender, short	Full	Large.
730-24	Small	Parsnip	Slender	Very full	Small.
898-24	Somewhat large	do	Slender, short	Decidedly full	Do.
992s-25	Decidedly small	Top	Slender	Full	Medium.
1161-24	Small	do	Slender, short	Semidepressed	Small.
1228s-25	do	do	Decidedly short	Full	Very small.
1293s-25	do	do	Short	Decidedly depressed.	Large.
1427-24	Large	do	Slender	Somewhat full	Small.
1569-24	Small	Parsnip	Slender, short	Very full	Somewhat small.
1591-24	Large	Top	Slender	Full	Small.
1612-24	do	do	do	do	Very small.
1818s-25	Medium	Turnip-parsnip	Short	Depressed	Very large.
1845s-25	do	Top	Slender, short	do	Somewhat large.
2287-24	Very large	Mangel-top	Slender	Full	Very small.
2310s-25	Decidedly large		Very slender	Semidepressed	Somewhat large.
2346s-25	Medium	Top	Slender, short	Somewhat full	Somewhat small.
2445s-25	do	Mangel-top	do	Very full	Very small.
3403-24	Somewhat large	Top	do	Semidepressed	Medium.
3956-24	Medium	Parsnip	Short	do	Do.
4545-24	Somewhat small	Top	Very slender	Depressed	Very large.
4621-22	Small	do	Decidedly slender	do	Do.
5656-24	Medium	do	Slender, short	Full	Medium.
5942-24	do	Mangel-top	Short	Very full	Very small.
6077-24	Somewhat large	Top	Decidedly slender	Full	Small.
7794-24	Medium	do	Short	Semidepressed	Medium.
8146-24	Large	Parsnip	do	Full	Small.
8753-24	Medium	Top	do	Semidepressed	Medium.

The absence of detailed figures in the published reports makes it impossible for other workers in the field to test the results, and the reader is deprived of the opportunity to check the conclusions of the writer or to make his own deductions. For this reason the results of the important morphological and anatomical measurements made in this study are given in detail for 20 of the 30 selections studied. (Table 2.) The mean, standard deviation, and coefficient of variability of some of the important characters are also shown. (Table 3.)

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928*

[Diameters were measured in the neck region (the smooth zone just below the leaf scars). Selections listed in numerical order]

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
550–24:	*Per cent*	*Grams*	*Mm.*	*Mm.*	*Mm.*	*Number*		*Mm.*	*Number*	
1927	11.3	1,193	110	31	10	11	2.0	8	160	Absent.
	11.4	795	90	29	7	10	2.2	6	100	Do.
	11.7	907	95	27	6	10	1.9	6	178	Present.
	11.8	907	90	29	8	9	1.8	8	188	Do.
	12.1	907	103	23	6.5	10	2.0	8	200	Absent.
	12.7	907	90	20	5	9	2.1	7	200	Present.
	13.0	907	85	25	7	8	2.1	7	120	Absent.
	13.1	738	85	20	5	9	2.2	6	200	Present.
	13.5	680	80	26	7	9	2.2	8	120	Absent.
	13.5	958	103	30	9	10	2.0	8	130	Do.
	13.7	1,079	103	26	6.5	10	1.9	8	154	Present.
	13.8	568	80	20	3.5	9	2.3	6	154	Absent.
	13.9	1,022	93	22	5	13	2.4	7	193	Do.
	14.0	596	80	20	4	9	2.4	5	169	Do.
	14.1	852	93	20	5	12	2.5	7	169	Do.
	14.3	680	80	21	5.5	11	2.7	6	170	Do.
	14.5	738	88	23	5	11	2.3	6	129	Do.
	14.5	795	85	25	5	10	2.3	7.5	110	Do
	14.7	1,079	100	23	4	10	2.2	7	168	Do.
	14.7	795	90	23	4	11	2.6	7	142	Present.
	15.1	680	80	24	6	9	2.3	5.5	138	Absent.
	15.1	738	80	21	6	11	2.7	5	154	Do.
	15.2	958	100	20	3.5	11	2.3	7.5	171	Do.
	15.4	568	75	20	4.5	9	2.2	6	154	Do.
	15.8	680	88	18	3	13	2.7	6	130	Do.
1928	15.4	680	90	20	3	10	2.1	------	170	
	16.1	1,052	105	22	5	9	1.8	------	140	Present.
	16.6	738	93	23	4.5	9	1.9	------	160	
	16.8	738	83	20	4	10	2.2	------	130	Do.
	17.0	1,022	97	20	2.5	11	2.1	------	190	
	17.4	1,022	72	22	3	11	2.1	------	150	
	17.4	882	90	22	3	10	2.2	------	120	Do.
	17.5	738	90	21	4	14	2.6	------	160	
	17.6	738	85	23	4.5	10	2.1	------	150	
	17.6	680	80	23	4.5	9	2.2	------	150	
	17.8	1,079	92	19	3	10	1.9	------	150	Do.
	17.8	768	96	16	4	12	2.3	------	130	Do.
	18.2	769	85	21	5	11	2.4	------	130	
	18.2	768	92	19	3	11	2.4	------	140	
	18.5	680	91	18	3	10	2.2	------	140	Absent.
	18.6	768	92	23	5	12	2.3	------	140	
	18.8	1,022	100	16	2.5	10	1.9	------	200	
	19.8	710	90	22	5	10	2.2	------	190	Do.
	20.2	907	95	17	2.5	10	2.0	------	180	Do.
	20.4	598	79	15	2	12	2.7	------	200	Do.
	(a)	710	85	18	3.5	12	2.4	------	------	
	(a)	882	90	24	5	11	1.9	------	------	
	(a)	483	70	14	2.5	10	2.3	------	------	
	(a)	795	95	23	4.5	11	2.0	------	------	
	(a)	907	95	16	2	13	2.2	------	------	

[a] Undetermined.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928*—Continued

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
554-24:	*Per cent*	*Grams*	*Mm.*	*Mm.*	*Mm.*	*Number*		*Mm.*	*Number*	
1927	11.0	1,477	103	19	2.5	10	1.9	9	153	Present.
	11.2	1,306	103	23	5	11	2.1	8	110	Do.
	11.4	625	75	22	6	10	2.6	4.5	185	Do.
	11.7	625	70	11	1.5	10	2.5	3.5	133	Do.
	12.2	907	85	20	4	9	2.1	6	172	Do.
	12.3	958	85	22	4	9	2.3	7	191	Do.
	12.4	738	78	17	2.5	11	2.3	7	161	Do.
	12.6	852	90	15	1.5	10	2.1	5	218	Do.
	13.4	907	93	18	2.5	9	1.9	7	166	Present.
	13.8	511	75	12	2	9	2.6	5	227	Absent.
	13.8	1,079	95	17	3.5	8	1.7	7	170	Do.
	13.9	958	95	18	3	8	1.7	7	200	Do.
	14.1	852	90	23	2.5	8	1.7	7	142	Present.
	14.1	738	85	13	1.5	10	2.3	5	180	Absent.
	14.1	1,134	100	25	4	11	2.1	5	144	Do.
	14.2	852	83	16	2.5	9	2.2	6	158	Present.
	14.2	680	80	16	3	11	3.0	4	133	Absent.
	14.3	795	90	20	4	10	2.1	7	200	Do.
	14.5	680	80	20	4	8	2.1	6	161	Do.
	14.5	795	90	20	3	9	2.0	5	141	Do.
	14.8	568	73	19	1.5	11	2.8	4	154	Do.
	14.9	795	75	17	3	12	2.8	5	133	Do.
	15.1	958	93	17	3	10	2.0	7.5	181	Do.
	16.1	625	80	18	2	10	2.4	4	161	Present.
	16.2	511	70	15	2.5	10	3.0	4	158	Absent.
1928	16.2	680	76	14	2	9	2.3	.....	160	Do.
	16.2	795	77	15	2	9	2.3	.....	.....	
	16.8	680	85	16	2	11	2.2	.....	160	Do.
	17.0	937	76	16	5	13	3.0	.....	160	Present.
	17.2	795	83	14	2.5	9	2.0	.....	160	Absent.
	17.4	907	90	14	2	11	2.2	.....	150	Do.
	17.6	937	86	15	2.5	12	2.6	.....	160	Do.
	17.8	882	86	15	2	11	2.4	.....	180	
	17.8	1,250	90	16	2	12	2.4	.....	.....	
	17.8	795	80	15	2	13	2.9	.....	.....	
	18.0	680	77	18	4	9	2.3	.....	170	Do.
	18.2	1,052	83	17	3.5	11	2.7	.....	160	
	18.2	937	82	15	2.5	10	2.2	.....	.....	
	19.0	882	83	11	1.5	12	2.6	.....	.....	
	19.2	680	74	22	6	10	2.4	.....	170	Do.
	19.4	852	85	14	2	12	2.6	.....	160	Present.
	19.4	710	83	15	2	11	2.6	.....	.....	
	19.8	825	80	14	3	12	2.9	.....	210	Do.
	20.2	907	85	18	3.5	13	2.9	.....	150	
	20.4	710	80	15	2	11	2.5	.....	.....	
	(a)	768	79	16	1.5	9	2.3	.....	.....	
	(a)	625	76	11	2	12	2.9	.....	.....	
	(a)	825	80	14	2	10	2.2	.....	.....	
	(a)	1,109	83	17	2	11	2.4	.....	.....	
	(a)	598	72	19	2	8	2.3	.....	.....	
600s-25:										
1927	9.0	568	83	24	6	8	1.9	7	157	Do.
	10.3	958	100	21	5	10	2.1	7	170	Do.
	10.8	852	98	21	5	12	2.4	7	118	Absent.
	10.8	758	85	20	3	9	2.1	5	133	Present.
	11.1	958	90	28	7.5	9	1.9	7	142	Absent.
	11.1	958	103	21	3	11	2.1	6	145	Do.
	11.3	680	93	26	9	9	2.0	5	139	Do.
	11.5	1,022	105	22	4	13	2.6	7	100	Do.
	11.7	852	95	28	9	8	1.9	7	100	Present.
	12.0	958	103	25	5.5	10	2.0	7	133	Absent.
	12.2	1,250	105	22	4	11	2.0	6	130	Present.
	12.2	1,022	110	24	8	10	1.7	6	110	Absent.
	12.2	1,022	95	17	3	13	2.5	7	110	Present.
	12.3	625	78	17	4	10	2.4	7	100	Absent.
	12.3	1,193	103	27	9	10	2.1	9	150	Do.
	12.4	907	90	16	3	11	2.4	6	150	Do.
	12.4	852	98	16	3	11	2.7	5	158	Do.
	13.2	1,193	105	22	3	12	2.3	6	100	Present.
	13.3	738	93	20	5	11	2.3	7	180	Do.
	13.5	568	70	22	8	8	2.0	5	142	Absent.

[a] Undetermined.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928*—Continued

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
600s-25—Contd.	*Per cent*	*Grams*	*Mm.*	*Mm.*	*Mm.*	*Number*		*Mm.*	*Number*	
1927	13.5	1,420	103	25	5.5	11	2.2	8	131	Absent.
	13.8	625	83	20	4.5	10	2.4	7	140	Do.
	13.8	738	85	17	5	12	2.5	6	191	Do.
	14.0	958	103	18	3	12	2.2	7	150	Do.
	14.0	852	85	27	7	9	2.0	7.5	175	Do.
1928	12.8	738	96	22	6.5	9	1.7	.....	140	Present.
	13.5	1,193	112	24	6	10	1.7	.....	140	Absent.
	13.6	988	102	23	5.5	11	1.9	.....	150	
	14.6	907	100	27	7	10	2.0	.....	80	
	15.2	937	93	21	7	13	2.3	.....	160	
	15.4	1,134	105	23	3.5	11	1.8	.....	130	Present.
	15.4	710	85	17	3.5	11	2.2	.....	150	
	15.5	1,022	92	26	7	10	1.8	.....	140	Absent.
	15.6	1,022	99	24	6	14	2.4	.....	140	
	15.6	907	86	22	4	9	2.0	.....	110	Do.
	16.0	852	96	20	2.5	11	2.1	.....	100	
	16.0	988	83	19	3.5	11	2.1	.....	140	
	16.0	937	102	25	6	10	1.7	.....	110	
	16.8	1,022	95	22	3.5	10	2.0	.....	130	
	16.8	1,052	105	20	3.5	12	2.1	.....	140	Do.
	17.5	1,134	205	19	3.5	12	1.8	.....	210	Do.
	17.5	795	97	14	3	13	2.5	.....	100	
	17.6	907	88	25	6	12	2.2	.....	170	
	18.0	958	90	17	4	10	2.3	.....	150	
	18.0	852	90	19	2.5	10	2.1	.....	150	
	(a)	907	95	23	5	10	1.9	.....	.....	Do.
	(a)	1,022	100	22	3.5	11	1.9	.....	.....	
	(a)	907	99	25	6	11	2.2	.....	.....	Do.
	(a)	852	99	20	3	12	2.0	.....	.....	
	(a)	598	85	20	4	11	2.4	.....	.....	Do.
808-24:										
1927	11.5	1,079	95	21	3	10	2.0	8	121	Do.
	11.8	1,022	98	24	5	11	2.4	7	170	Do.
	12.0	907	95	24	7	10	2.2	6.5	112	Do.
	12.1	1,079	95	17	4	11	2.2	6.5	190	Do.
	12.3	852	80	15	4	10	2.4	6.5	135	Do.
	12.5	1,022	103	25	7	10	2.0	6	144	Do.
	12.6	1,079	95	21	3	12	2.2	7	137	Do.
	12.6	738	80	19	6	12	2.6	6	112	Do.
	12.8	1,306	103	24	7	11	2.1	9	121	Do.
	13.0	958	88	25	8	9	2.1	7	137	Do.
	13.0	680	80	22	9	11	2.6	6	112	Do.
	13.2	625	75	21	4	10	2.4	7	157	Do.
	13.4	1,022	90	24	6	11	2.2	7	137	Do.
	13.5	738	80	17	2	10	2.6	5.5	129	Do.
	13.6	1,079	93	20	2	11	2.3	7	130	Do.
	13.6	852	85	27	10	8	1.9	7	98	Do.
	14.2	958	90	27	11	11	2.3	3.5	137	Do.
	14.3	907	90	24	4	10	2.2	6.5	121	Do.
	14.3	680	83	18	6	11	2.4	5	119	Do.
	14.6	1,079	95	17	2	10	2.1	6	200	Do.
1928	13.5	825	90	26	7	11	2.44	6	110	Present.
	14.2	765	80	18	3.5	10	2.00	7	89	Absent.
	13.4	907	102	23	8	10	1.85	7	87	Do.
	15.4	935	92	23	5	10	2.04	7	146	Do.
	15.8	985	90	19	5	10	2.00	6.5	106	Do.
	16.0	1,193	104	25	5	11	2.00	7	130	Present.
	16.4	958	90	20	5	12	2.40	7	113	Absent.
	16.4	907	87	17	3	12	2.50	6	140	Do.
	16.5	852	90	16	3	12	2.35	6	140	Do.
	16.5	935	92	19	4	12	2.31	6.5	105	Do.
	17.0	738	80	20	3.5	11	2.60	7	100	Do.
	17.1	680	80	20	3.5	10	2.32	6.5	132	Do.
	17.0	795	85	20	4	11	2.44	6	100	Do.
	17.5	1,134	97	20	4	11	2.07	8	131	Do.
	17.6	795	80	16	4	12	2.35	6	100	Do.
	18.2	985	82	20	5	12	2.40	6	112	Do.
	18.2	825	90	17	5	12	2.35	7	134	Do.
	18.2	710	77	17	4	10	2.08	5	125	Do.
	18.3	852	87	18	5	9	1.91	8	140	Do.
	18.4	907	92	22	6	11	2.07	8	145	Do.
	18.6	958	87	20	5	11	2.11	6	125	Present.

a Undetermined.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928*—Continued

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
898-24—Contd.	*Per cent*	*Grams*	*Mm.*	*Mm.*	*Mm.*	*Number*		*Mm.*	*Number*	
1928	18.6	1,361	100	21	6	12	2.10	7	97	Absent.
	19.0	680	75	17	4	11	2.56	6	146	Do.
	19.3	852	90	18	3.5	11	2.11	7	144	Do.
	19.5	680	80	17	4	12	2.61	6	125	Do.
1161-24:										
1927	10.7	625	85	17	4	9	2.1	6.5	166	Do.
	11.1	680	83	15	3	10	2.2	5	142	Present.
	11.3	795	90	19	3	9	2.0	6	166	Absent.
	11.4	958	85	22	6.5	10	2.3	5.5	90	Do.
	11.8	680	85	17	3	10	2.4	5	116	Do.
	12.1	1,590	113	22	6.5	10	1.8	7.5	210	Do.
	12.1	1,134	98	22	6.5	13	2.4	6	133	Present.
	12.4	625	75	17	3.5	9	2.4	7	146	Do.
	12.4	738	85	19	5.5	8	1.9	5	131	Absent.
	12.5	1,079	95	24	6.5	10	2.0	7	133	Do.
	12.7	907	83	21	6	12	2.7	5.5	120	Do.
	12.8	1,250	110	21	5	10	2.0	8	200	Do.
	12.8	680	78	20	7	9	2.4	5	121	Present.
	12.8	852	83	20	4.5	9	2.1	6.5	190	Absent.
	12.9	958	93	25	8	10	2.1	6	142	Present.
	13.1	907	90	19	4	9	1.9	6.5	182	Absent.
	13.2	795	80	23	6	8	1.7	7	170	Do.
	13.2	680	83	19	3	10	2.1	5	141	Present.
	13.3	958	87	20	5	11	2.3	6	163	Do.
	13.3	958	90	21	5	9	2.0	7	161	Do.
	13.5	1,022	95	20	4	9	1.8	8	200	Do.
	13.6	907	93	20	3	9	2.0	4	150	Absent.
	13.8	958	93	19	4	10	2.0	7	133	Present.
	13.9	680	85	15	3	10	2.4	5	200	Absent.
	15.0	680	80	21	5	10	2.4	6	146	Do.
1928	15.6	1,022	94	15	3	11	2.2	.....	140	Present.
	16.4	710	85	17	2.5	10	2.1	.....	150	Absent.
	16.6	882	85	16	3	10	2.1	.....	140	Do.
	16.6	541	75	15	2	10	2.4	.....	150	
	17.1	680	83	14	2.5	10	2.0	.....	110	Do.
	17.2	541	78	14	2	9	2.0	.....	140	Do.
	17.8	937	90	17	3	10	1.9	.....	190	Present.
	17.4	852	84	19	3.5	8	2.1	.....	130	Absent.
	17.4	795	87	15	2.5	10	2.0	.....	160	
	17.5	710	77	16	3	10	2.3	.....	140	
	17.7	907	84	13	2	9	1.9	.....	140	
	17.9	710	80	14	2	10	2.0	.....	150	
	18.2	958	86	18	3	10	1.9	.....	130	
	18.2	568	80	14	2	10	2.2	.....	150	
	18.3	795	88	16	2.5	10	1.9	.....	150	Do.
	18.4	568	73	11	1.5	9	2.2	.....	160	Do.
	18.4	680	80	12	2	10	2.3	.....	180	
	18.5	738	85	14	2	10	2.0	.....	150	Do.
	18.8	680	80	15	2.5	9	1.9	.....	140	
	19.4	795	83	15	2.5	9	2.0	.....	120	
	(a)	795	85	.....	.....	.....	.....	.....	.....	
	(a)	625	74	18	4	9	2.1	.....	.....	
	(a)	483	79	11	2	10	2.3	.....	.....	
	(a)	458	66	13	2	10	2.6	.....	.....	
	(a)	483	70	15	3	9	2.1	.....	.....	
1228s-25:										
1927	9.7	1,079	110	23	5	10	1.8	6	158	Present.
	10.1	1,306	110	23	5	10	1.8	8	118	Do.
	10.5	1,134	105	27	5	9	1.7	7	166	Absent.
	10.6	568	80	23	6	9	2.1	6	200	Do.
	11.4	511	80	20	3.5	9	2.1	5	133	Do.
	11.6	855	90	25	5	9	1.8	7	150	Do.
	11.7	852	90	23	5	9	1.9	6	125	Do.
	11.7	738	95	20	4	10	2.0	5	145	Do.
	12.0	625	90	26	6	9	2.0	6	130	Do.
	12.0	680	90	23	5	8	1.7	7	136	Do.
	12.1	738	100	25	4	8	1.7	7	200	Do.
	12.3	958	90	26	6	10	2.1	7	154	Do.
	12.5	958	95	20	2.5	10	2.1	7	125	Do.
	12.5	958	85	23	5	9	1.8	7	163	Do.
	12.7	852	100	20	3.5	11	2.2	5	125	Do.
	12.8	568	83	23	2.5	9	2.1	6	175	Do.

[a] Undetermined.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928*—Continued

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
1228s–25—Contd.	*Per cent*	*Grams*	*Mm.*	*Mm.*	*Mm.*	*Number*		*Mm.*	*Number*	
1927	13.0	907	100	23	4	8	1.7	6	133	Absent.
	13.0	1,195	110	25	-------	10	1.8	7	133	Do.
	13.2	1,195	110	24	6	11	2.0	6	145	Present.
	13.2	453	80	23	4.5	7	1.8	6	142	Absent.
	13.3	738	98	23	5	10	2.0	5	118	Do.
	13.8	625	83	20	4	10	2.3	4	166	Do.
	13.9	958	90	21	5	11	2.1	5	140	Do.
	13.9	1,022	100	25	7	9	1.7	6	175	Present.
	14.4	852	85	20	4	10	2.3	6	163	Absent.
1928	15.0	710	85	27	5	9	1.98	7	89	
	15.0	852	95	18	3	10	2.00	7	110	
	15.4	795	90	20	3	9	1.87	5	109	
	15.4	710	85	17	3	9	1.73	5	204	
	15.4	680	84	19	4	10	2.13	5	100	
	15.7	882	101	20	3.5	10	1.85	6	175	
	15.8	1,022	91	18	3	10	1.98	6	80	
	15.8	1,022	102	25	5	10	1.80	6.5	96	
	16.0	768	88	16	2	10	2.08	7	87	
	16.0	568	85	20	3	9	2.14	6	123	
	16.2	595	85	20	3	9	1.80	4	96	
	16.2	880	102	21	3.5	9	1.61	5	195	
	16.6	595	85	20	4	9	2.00	4	113	
	16.6	795	95	19	3	9	1.80	6	187	
	17.0	852	92	20	4.5	10	1.89	6	105	
	17.0	795	96	18	3	11	2.00	5	123	
	17.0	880	94	14	2	12	2.40	7	80	
	16.8	670	85	20	4	8	1.54	4	80	
	17.5	680	95	19	3.5	10	2.08	6	105	
	17.6	764	90	23	4.5	10	2.13	8	136	
	17.8	680	83	22	5	9	1.87	6	104	
	17.8	680	80	15	1.5	10	2.13	4	108	
	17.9	795	97	25	4.5	10	1.85	6.5	139	
	18.6	768	90	20	4.5	10	2.08	6	123	
	18.6	738	82	12	1.5	11	2.24	7	139	
1427–24:										
1927	11.5	1,079	95	21	3	10	2.0	8	141	Do.
	11.8	1,022	98	24	5	11	2.4	7	170	Present.
	12.0	907	95	24	7	10	2.2	6.5	124	Absent.
	12.1	1,079	95	17	4	11	2.2	6.5	118	Do.
	12.3	852	80	15	4	10	2.4	6.5	194	Do.
	12.5	1,022	103	25	7	10	2.0	6	141	Do.
	12.6	1,079	95	21	3	12	2.2	7	147	Do.
	12.6	738	80	19	6	12	2.6	7	124	Present.
	12.8	1,306	103	24	7	11	2.1	9	130	Absent.
	13.0	958	88	25	8	9	2.1	7	153	Do.
	13.0	680	80	22	9	11	2.6	6	159	Do.
	13.2	625	75	21	4	10	2.4	7	182	Do.
	13.4	1,022	90	24	6	11	2.2	7	153	Do.
	13.5	738	80	17	2	10	2.6	5.5	153	Do.
	13.6	1,071	93	20	2	11	2.3	7	141	Present.
	13.6	852	85	27	10	8	1.9	7	130	Absent.
	14.2	958	90	27	11	11	2.3	7.5	159	Do.
	14.3	907	90	24	4	10	2.2	6.5	147	Present.
	14.3	680	83	18	6	11	2.4	5	159	Absent.
	14.6	1,079	95	17	2	10	2.1	6	279	Do.
1928	15.4	738	78	18	2	10	2.30	-------	150	
	16.8	568	87	23	4	11	2.10	-------	160	
	16.8	1,193	74	16	2	10	2.40	-------	160	
	16.8	680	69	18	3	11	2.90	-------	200	
	17.0	795	73	13	2	11	2.60	-------	180	
	17.2	598	65	16	2.5	11	2.90	-------	130	
	17.2	710	69	14	2	10	2.50	-------	180	
	17.2	511	66	14	2.5	12	3.30	-------	130	
	17.2	680	76	20	3.5	8	1.90	-------	170	
	17.8	768	75	16	2.5	11	2.50	-------	180	
	17.5	710	80	20	3.5	10	2.20	-------	160	
	17.5	795	75	16	2.5	10	2.50	-------	180	
	17.8	680	73	16	2	10	2.30	-------	140	
	17.8	680	68	12	2	10	2.50	-------	120	
	18.2	768	79	13	3	11	2.30	-------	240	
	18.5	625	75	16	2	11	2.50	-------	100	
	18.6	907	87	18	2	12	2.50	-------	160	
	18.6	795	83	16	2.5	12	2.30	-------	220	

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928*—Continued

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
1427-24—Contd.	*Per cent*	*Grams*	*Mm.*	*Mm.*	*Mm.*	*Number*		*Mm.*	*Number*	
1928	18.6	680	72	18	5	9	2.30	.....	120	
	18.8	710	80	18	3	11	2.60	.....	170	
	(a)	680	75	19	3	10	2.40	.....	.....	
	(a)	568	68	25	6.5	8	2.10	.....	.....	
	(a)	625	70	16	3	.....	2.80	.....	.....	
	(a)	568	73	22	6	.....	2.60	.....	.....	
	(a)	423	60	11	2	.....	3.00	.....	.....	
1569-24:										
1927	9.6	1,134	95	22	6	9	1.7	7	172	Absent.
	9.8	852	88	30	10	9	2.0	4.5	190	Do.
	10.5	625	80	18	5	10	2.2	6	153	Do.
	10.7	680	83	22	6	9	2.3	4	200	Do.
	10.7	738	83	20	4	10	2.7	5	108	Do.
	10.7	958	85	26	10	10	1.9	6	126	Do.
	10.8	795	85	23	5	9	2.2	6	114	Do.
	11.2	738	85	20	5	9	2.2	6	175	Do.
	11.3	1,079	93	22	5.5	10	2.1	5	180	Do.
	11.7	568	80	26	6	11	2.5	5	100	Do.
	11.8	625	78	22	6	9	2.6	5.5	113	Do.
	11.8	795	85	22	6	10	2.4	5	137	Do.
	12.0	680	95	20	5.5	10	1.9	6	144	Do.
	12.0	738	88	20	5	10	2.1	6.5	119	Do.
	12.5	738	95	22	6	10	2.0	5	142	Do.
	12.6	738	88	23	7	11	2.2	6	200	Do.
	12.8	511	68	23	4	9	2.4	5	147	Do.
	12.8	625	88	18	5	10	2.3	5	160	Do.
	12.8	738	83	23	7	10	2.3	5	100	Do.
	13.5	511	78	19	5	10	2.6	5	110	Do.
1928	10.2	935	90	21	4	11	2.20	5	140	
	12.4	985	90	23	6.5	11	2.34	7	110	Do.
	12.4	825	85	22	6	11	2.39	5	160	
	12.8	985	95	20	4	11	2.20	7	133	
	12.9	907	90	26	6	11	2.15	6	105	
	13.0	825	90	18	3	11	2.13	6	170	Do.
	13.2	795	92	18	3	11	2.08	5	120	Do.
	13.2	768	90	22	5.5	12	2.26	5	105	
	13.5	985	95	22	4	12	2.20	6	166	
	13.5	796	90	20	3	11	2.29	6	110	
	13.8	1,079	92	20	4	11	2.08	6	130	
	14.0	985	92	22	6	10	1.85	6	150	Do.
	14.0	985	93	21	5	11	2.11	6	155	Do.
	14.0	738	90	21	3.5	10	2.00	5	133	
	14.3	825	90	18	3	11	2.16	5.5	210	
	14.6	825	90	20	3	10	1.92	6.5	155	
	14.6	935	97	20	3	11	2.04	7	110	Do.
	14.8	825	88	24	6	12	2.35	5.5	112	
	14.6	907	90	19	4.5	11	2.20	7	112	Do.
	14.7	1,022	92	28	7	11	2.04	7	140	
	14.9	595	78	18	4	10	2.27	5.5	130	Do.
	15.2	765	87	21	5	11	2.08	7	140	
	15.8	710	87	22	5.5	10	2.17	6	103	
	17.8	907	90	18	4	10	2.04	6	150	
	(a)	765	87	18	2.5	12	2.60	6	155	Do.
1612-24:										
1927	9.5	738	90	27	7.5	9	2.0	7	153	Do.
	9.9	795	95	22	4	10	2.1	7	130	Do.
	10.2	852	90	21	5	11	2.2	6.5	206	Do.
	10.5	852	93	20	4	11	2.2	7.5	125	Do.
	11.8	795	85	20	4	11	2.5	6	162	Do.
	12.2	738	88	25	7	10	2.3	8	100	Do.
	12.6	852	95	17	3	10	2.1	7	188	Do.
	12.6	1,079	100	20	3.5	12	2.3	7	188	Do.
	12.6	1,079	95	17	4	12	2.3	7	124	Do.
	12.8	1,134	95	23	4	10	1.9	7	90	Do.
	13.0	680	78	24	7.5	9	2.3	6.5	188	Do.
	13.0	625	90	20	5	10	2.0	7	141	Do.
	13.0	795	85	20	5	9	2.0	7.5	206	Do.
	13.2	1,079	95	20	6	11	2.3	7	150	Do.
	13.2	958	98	17	3	13	2.6	6	118	Do.
	13.5	680	85	19	5	11	2.4	5.5	100	Do.
	13.8	738	80	17	3	12	2.8	5	112	Do.
	14.0	738	83	17	2.5	11	2.4	5	135	Do.
	14.3	625	75	20	3	9	2.3	7	159	Do.
	14.4	625	83	22	8	10	2.3	4	141	Do.

(a) Undetermined.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928*—Continued

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
1612-24—Contd.	*Per cent*	*Grams*	*Mm.*	*Mm.*	*Mm.*	*Number*		*Mm.*	*Number*	
1928	15.0	1,022	100	18	3	11	2.1	.....	190	Absent.
	15.0	825	85	23	3.5	10	1.7	.....	120	
	16.2	907	95	23	3	9	1.9	.....	160	
	16.2	882	90	23	4	10	2.0	.....	130	Present.
	17.0	937	95	18	2.5	10	2.1	.....	140	
	17.0	907	87	21	4	10	2.2	.....	150	Do.
	17.2	1,079	99	22	5	12	2.4	.....	110	
	17.2	1,079	93	23	3	11	2.2	.....	150	
	17.4	680	87	18	2	12	2.4	.....	160	
	17.6	882	100	16	2	11	2.1	.....	110	
	17.7	738	83	18	4	12	2.7	.....	140	
	18.0	882	90	14	2	11	2.2	.....	160	
	18.2	710	80	13	2	11	2.5	.....	190	
	18.3	882	89	16	3	11	2.1	.....	160	Absent.
	18.6	907	88	22	6.5	11	2.2	.....	150	Do.
	18.6	680	78	18	5	11	2.4	.....	160	Do.
	18.6	710	87	16	2	10	2.1	.....	150	
	19.0	1,134	104	18	3.5	13	2.3	.....	170	
	19.6	852	95	19	4	11	2.2	.....	210	Do.
	20.0	738	90	15	2	11	2.2	.....	150	
	(a)	625	90	18	3	11	2.3	.....	.....	
	(a)	958	97	20	3	10	2.0	.....	.....	
	(a)	882	90	15	2	12	2.3	.....	.....	
	(a)	710	93	15	2.5	12	2.3	.....	.....	
	(a)	710	90	20	3	10	2.2	.....	.....	
1845s-25:										
1927	11.2	1,361	110	19	3	10	1.8	7	131	Do.
	12.5	1,134	95	20	3	10	2.0	8	144	Do.
	12.6	795	88	21	3	10	2.2	7	100	Do.
	12.9	738	80	20	3	10	2.6	8	137	Do.
	12.9	852	84	19	5.5	10	2.3	7	106	Do.
	13.5	680	83	22	3	10	2.4	5	119	Do.
	13.5	958	93	20	4	10	2.0	8	119	Do.
	13.6	680	80	17	3	10	2.4	6	87	Do.
	13.6	958	100	24	3.5	11	2.3	7	100	Do.
	14.0	738	82	19	4	11	2.6	6	113	Do.
	14.1	907	95	17	2	10	2.0	5.5	106	Do.
	14.1	852	90	19	3	11	2.2	7	125	Do.
	14.3	680	82	18	3	9	2.2	6.5	113	Do.
	14.5	795	93	20	3	10	2.1	7	131	Do.
	14.6	738	90	19	2	10	2.0	6	94	Do.
	14.8	738	84	19	3	9	2.1	8	144	Do.
	14.8	625	73	12	1	13	3.4	5	100	Do.
	15.0	852	85	22	4	9	2.2	8	193	Do.
	15.0	852	85	11	1.5	12	2.6	5.5	119	Do.
	15.8	852	95	13	3	14	2.9	7	144	Do.
2287-24										
1927	11.4	1,134	105	30	9	10	2.0	7	161	Do.
	11.6	795	80	27	6.5	8	2.0	8.5	195	Do.
	12.0	738	85	23	6	10	2.3	7	150	Present.
	12.0	738	85	26	6	8	1.9	7	172	Absent.
	12.2	738	80	20	5	9	2.2	6	140	Do.
	12.5	852	93	28	6	9	2.0	8	131	Present.
	12.7	958	90	26	5	9	2.0	6	150	Absent.
	12.8	852	90	30	10	9	2.1	5.5	121	Do.
	12.8	680	80	22	5	9	.....	7	192	Do.
	12.8	738	85	30	7	8	2.0	7	121	Do.
	13.0	958	105	26	5	10	1.8	4	142	Do.
	13.2	625	80	20	5	10	2.4	.....	.....	
	13.3	907	88	38	14	9	1.8	7	136	Do.
	13.3	738	80	28	6	8	1.9	7	164	Do.
	13.4	625	75	17	2	11	2.8	5	163	Do.
	13.6	958	95	26	8	10	2.1	8	138	Do.
	13.8	1,134	95	32	6	9	2.1	6	142	Do.
	14.0	795	90	22	5	12	2.6	7	182	Do.
	14.0	1,022	95	30	8	10	1.9	6	134	Do.
	14.0	625	80	27	6	8	1.9	6	166	Do.
	14.1	738	90	24	5	9	2.0	5	158	Do.
	14.6	1,022	90	23	5	11	2.2	6	191	Present.
	14.6	625	80	22	5	9	2.2	6	138	Do.
	14.7	795	85	27	6	9	2.0	4	182	Absent.
	14.9	795	83	22	5	9	2.1	7	142	Do.

[a] Undetermined.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928*—Continued

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
	Per cent	*Grams*	*Mm.*	*Mm.*	*Mm.*	*Number*		*Mm.*	*Number*	
2310s-25: 1927	11.1	1,134	98	33	12	10	2.1	6	192	Present.
	12.1	907	100	22	3.5	14	3.0	6	141	Absent.
	12.3	1,361	115	24	5	13	2.4	5	183	Do.
	12.4	1,022	98	22	7	10	2.0	7	190	Do.
	12.5	625	88	20	5	10	2.2	4	120	Do.
	12.6	1,079	100	28	7	10	2.0	7	179	Do.
	13.0	1,079	100	20	3.5	8	2.1	7	170	Do.
	13.0	1,306	110	28	4	11	1.9	9	180	Do.
	13.1	907	85	20	4	12	2.6	6	143	Present.
	13.2	937	90	15	4	9	2.0	6	216	Do.
	13.3	907	100	23	6	10	2.2	7	123	Absent.
	13.3	625	83	24	8	8	2.2	6	200	Do.
	13.4	734	95	22	6	11	2.3	6	223	Do.
	13.5	738	93	20	6	10	2.2	5.5	141	Do.
	13.6	907	100	23	6	10	2.2	4.5	150	Do.
	13.8	738	95	19	3	10	2.3	5	193	Present.
	13.8	738	99	20	5	11	2.5	6	120	Absent.
	14.0	1,134	115	22	5	12	1.7	6	115	Do.
	14.1	625	88	18	4	9	2.5	7	157	Do.
	14.1	907	95	22	5	11	2.3	7	140	Do.
	14.1	1,022	100	27	7	13	2.6	4	143	Do.
	14.4	1,022	100	19	5	13	2.7	6	158	Do.
	14.5	907	95	21	3.5	10	2.2	7	190	Do.
	14.8	1,079	103	24	7	11	2.2	6	161	Do.
	15.0	958	98	15	3	13	2.5	7	140	Do.
2445s-25: 1927	12.5	1,022	98	17	3	10	2.2	8	200	Present.
	12.7	917	95	24	5	10	1.7	8	200	Do.
	12.7	568	75	25	7.5	9	2.4	7	157	Absent.
	12.9	680	75	22	3.5	9	2.3	6	213	Present.
	13.1	907	93	26	4	11	2.5	7	177	Do.
	13.2	680	73	18	2	9	2.4	5	210	Do.
	13.2	680	78	19	5	10	2.4	7	200	Do.
	13.3	1,420	100	21	3.5	12	2.3	7	177	Do.
	13.8	341	90	21	3	12	2.5	5	210	Do.
	13.8	625	75	25	5	9	2.1	6	154	Do.
	13.8	907	85	18	2	9	1.9	7	110	Absent.
	14.1	568	73	20	3.5	9	2.6	7	200	Do.
	14.3	680	90	27	6.5	11	3.3	8	193	Present.
	14.3	958	85	30	8	8	2.0	7	228	Do.
	14.4	738	85	21	3.5	11	2.4	6.5	220	Absent.
	14.7	738	90	16	2	10	2.6	6.5	218	Do.
	14.7	852	90	18	2	10	2.3	7	110	Do.
	14.8	680	80	25	4.5	11	2.7	7	166	Present.
	15.0	738	78	17	3	12	2.8	6	163	Do.
	15.1	568	70	23	4.5	11	2.1	6	200	Do.
	15.1	1,079	90	16	2	11	2.3	6	169	Do.
	15.1	738	80	18	2	11	2.7	5	210	Do.
	15.6	568	70	22	3.5	9	2.6	5	166	Do.
	15.8	1,022	90	30	4.5	11	2.7	8	191	Do.
	15.8	738	80	16	2.5	14	3.4	4.5	150	Absent.
3403-24: 1927	8.3	962	88	20	4	10	2.3	5.5	133	Do.
	8.5	1,361	108	23	7	12	2.3	6	123	Present.
	9.0	1,250	90	22	5	10	2.1	6	142	Absent.
	9.5	1,193	95	19	4	12	2.4	4	133	Do.
	9.8	738	75	26	9	9	2.1	5	150	Do.
	9.8	1,306	100	27	9	11	2.4	6	150	Present.
	9.9	958	85	22	6	10	2.1	5	142	Absent.
	9.9	1,420	110	29	5	9	1.7	6	175	Do.
	9.9	958	90	13	3	9	2.4	4	190	Present.
	10.0	958	93	20	4	9	2.1	6.5	150	Absent.
	10.0	1,590	103	24	8	11	1.8	9.5	143	Present.
	10.2	1,134	80	20	5	11	2.6	6	210	Absent.
	10.5	1,079	100	29	9	10	2.1	6	116	Do.
	10.8	1,022	90	26	7	9	1.8	5	158	Do.
	11.0	558	90	22	8	10	2.2	4	139	Do.
	11.0	1,134	85	22	4	11	2.3	5	150	Do.
	11.0	738	80	22	4	8	2.0	6	131	Do.
	11.3	1,022	90	20	9	9	2.2	5	100	Do.
	11.7	738	80	20	5	11	2.7	5	150	Present.
	11.9	958	90	19	4	10	2.4	6	130	Do.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928*—Continued

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
3403-24—Contd	*Per cent*	*Grams*	*Mm.*	*Mm.*	*Mm.*	*Number*		*Mm.*	*Number*	
1927	12.1	680	80	23	6	9	2.3	5	140	Absent.
	13.0	852	80	23	7	8	2.0	5	180	Do.
	13.3	1,134	88	20	6	11	2.6	6	166	Do.
	13.6	852	85	20	3	9	2.3	7	210	Present.
	14.1	907	83	18	3	10	2.3	6	190	Absent.
1928	14.0	1,361	98	20	3.5	11	2.0	.....	90	Do.
	14.8	1,022	95	23	5	11	2.1	.....	140	
	16.4	625	75	17	2.5	10	2.1	.....	100	Present.
	16.8	825	80	17	4	9	2.2	.....	130	
	17.0	852	80	18	4	11	2.2	.....	90	Absent.
	17.0	1,022	94	19	4	12	2.3	.....	160	Present.
	17.0	907	82	20	3	11	2.4	.....	120	Do.
	17.2	655	70	18	4	9	2.3	.....	110	Absent.
	18.8	958	85	17	2.5	11	2.4	.....	130	Present.
	18.0	795	77	16	2.5	11	2.7	.....	130	
	18.0	795	78	19	4	9	2.1	.....	150	
	18.6	907	80	20	4	10	2.1	.....	150	Absent.
	20.0	541	70	12	1.5	11	2.7	.....	150	
	20.0	882	85	14	1.5	11	2.5	.....	140	
	20.2	710	77	10	1.5	12	2.7	.....	140	Do.
	(a)	1,079	90	22	6	10	1.9	.....	110	Do.
	(a)	655	76	19	5	11	2.5	.....	120	
	(a)	882	91	21	6	11	2.2	.....	120	
	(a)	680	75	18	4	9	2.2	.....	110	
	(a)	680	82	20	4	10	2.3	.....	120	
	(a)	511	74	11	1	12	2.6	.....	.....	
	(a)	568	72	12	2	13	3.0	.....	.....	
	(a)	483	67	12	1.5	11	3.2	.....	.....	
	(a)	795	76	14	1.5	11	2.4	.....	.....	
	(a)	453	67	14	2	10	3.0	.....	.....	
3956-24:										
1927	11.2	1,134	100	20	3.5	10	2.1	5	142	Do.
	11.5	1,306	103	20	4	10	2.2	5	110	Do.
	11.7	1,079	105	23	7	11	2.1	5	142	Do.
	11.8	680	80	20	5.5	10	2.5	6	125	Do.
	12.0	1,134	95	20	5	11	2.3	7	117	Do.
	12.2	1,818	115	25	7	12	2.0	7	110	Do.
	12.4	1,306	117	15	5	14	2.6	7	98	Do.
	12.5	852	88	19	4	15	3.0	6	105	Do.
	12.5	795	95	21	5	10	2.0	6	110	Do.
	12.6	1,306	108	21	4	14	2.5	6	117	Do.
	12.7	852	95	17	4	12	2.5	5	100	Do.
	12.8	958	100	15	2.5	11	2.1	6	177	Do.
	13.0	1,079	95	21	5	12	2.5	6	98	Do.
	13.2	1,134	95	25	7	12	2.3	5	80	Do.
	13.3	852	95	10	1.5	14	2.6	6	120	Do.
	13.4	907	85	16	3	14	3.2	5	102	Do.
	13.4	907	90	20	4	10	2.1	8	142	Do.
	13.5	1,134	103	19	4	12	2.4	6	148	Do.
	13.8	852	90	18	4	12	2.3	4	145	Do.
	14.0	907	95	13	3	12	2.6	4	148	Do.
	14.1	1,022	108	18	5	14	2.7	7	180	Do.
	14.6	958	93	17	5	13	2.7	6	132	Do.
	15.0	958	100	18	4	14	2.7	8	125	Do.
	15.8	795	90	15	3	14	3.0	5	140	Do.
	16.4	738	85	16	3	15	3.1	4	127	Do.
1928	15.2	710	90	9	4	17	3.15	5	100	
	15.8	1,022	105	23	5.5	16	2.71	7	71	
	16.2	795	90	21	6	13	2.55	5	134	
	16.3	958	100	15	2	14	2.33	7	108	
	16.6	680	87	18	3	12	2.50	6.5	72	
	16.8	907	102	18	3	12	2.18	7	94	
	17.0	907	95	17	2.5	16	3.02	6	79	
	17.6	1,022	105	18	3.5	14	2.41	7	66	
	17.6	795	95	15	2.5	13	2.45	6	107	
	18.0	907	94	16	2.5	11	2.08	7	132	
	18.0	680	87	17	3.5	14	3.04	6	80	
	18.0	680	90	13	3	14	2.45	6	100	
	18.0	795	87	17	3	13	2.76	6	89	
	18.2	795	95	15	2.5	15	2.83	6	104	
	18.2	738	90	12	2	16	3.13	6.5	116	
	18.4	680	87	13	2	14	2.58	5	130	

(a) Undetermined.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928*—Continued

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
3956-24—Contd.	*Per cent*	*Grams*	*Mm.*	*Mm.*	*Mm.*	*Number*		*Mm.*	*Number*	
1928	18.8	852	98	17	3.5	13	2.45	6.5	65	
	18.8	907	97	18	4	14	2.59	6	104	
	19.0	1,100	107	18	2.5	17	2.83	7	81	
	19.2	680	85	12	2.5	17	3.69	5	70	
	(*)	1,022	102	15	2.5	13	2.36	6.5	99	
	(*)	540	83	17	3.5	14	3.04	6	86	
	(*)	795	94	16	3	15	2.68	7	108	
	(*)	852	98	18	3	14	2.54	8	129	
	(*)	680	85	14	3.5	14	2.99	5	94	
4545-24:										
1927	10.1	958	85	14	2.5	13	2.6	7	183	Absent.
	11.1	568	73	22	5.5	11	2.8	4	145	Do.
	12.1	680	78	25	7.5	9	2.2	5	182	Present.
	12.3	453	68	23	6	10	2.8	5	166	Do.
	12.7	1,079	85	23	6	11	2.5	5	-------	Absent.
	13.1	1,022	83	26	6	9	2.1	6	200	Present.
	13.2	680	80	18	4.5	11	2.8	5	190	Do.
	13.2	511	65	17	4.5	12	3.2	5	136	Do.
	13.3	511	68	22	6	10	2.9	6	210	Do.
	13.4	795	80	22	4.5	9	2.1	7	150	Absent.
	13.5	1,079	88	21	3	10	2.3	6	180	Do.
	13.5	795	83	22	3.5	11	2.6	7	210	Do.
	13.7	625	75	24	9	12	3.1	4	166	Do.
	13.8	1,079	90	24	9	11	2.1	6	-------	Do.
	13.9	1,022	83	21	5.5	10	2.5	5	175	Do.
	14.1	568	68	-------	6.5	10	2.6	4	200	Present.
	14.3	580	73	16	2.5	11	3.0	4	173	Do.
	14.5	738	78	18	3	11	2.6	6	137	Do.
	14.7	738	75	19	3.5	12	2.8	5	245	Do.
	14.8	738	73	20	3	12	3.0	4	190	Do.
	14.8	852	90	19	5	15	3.2	4	120	Absent.
	15.3	852	73	20	3.5	12	2.9	4	166	Do.
	15.3	511	65	17	3.5	10	2.8	5	177	Do.
	16.0	568	65	22	5.5	12	3.4	3	144	Do.
	16.6	625	75	18	3	11	2.9	5	180	Do.
1928	15.2	655	70	18	2	13	3.1	-------	150	Present.
	15.4	907	85	25	3.5	11	2.5	-------	140	Do.
	15.4	655	72	23	6	10	2.4	-------	160	Do.
	15.4	1,022	77	17	2.5	10	2.3	-------	130	Do.
	15.8	680	70	18	4	10	2.3	-------	120	Absent.
	16.2	655	75	22	5	11	2.5	-------	140	Present.
	16.3	568	73	18	2.5	10	2.4	-------	130	Do.
	16.7	1,134	87	24	5.5	12	2.3	-------	120	Absent.
	16.9	935	80	22	3	14	2.8	-------	140	Present.
	17.0	825	75	23	5	11	2.4	-------	130	Do.
	17.0	880	80	17	2	12	2.4	-------	140	Do.
	17.5	710	70	14	2	12	2.8	-------	130	Do.
	17.6	907	87	21	4	15	3.0	-------	150	Do.
	17.6	598	72	22	3	10	2.4	-------	170	Do.
	18.0	680	68	20	5	13	3.2	-------	120	Do.
	18.0	880	72	22	4	12	2.6	-------	150	Absent.
	18.2	511	70	18	2.5	11	2.9	-------	180	Present.
	18.8	680	73	16	3	13	2.6	-------	110	Do.
	19.0	765	74	17	2.5	11	2.6	-------	170	Do.
	19.0	540	63	20	3	10	2.8	-------	140	Do.
	(*)	935	80	22	3	11	2.4	-------	140	
	(*)	625	63	16	5	12	2.6	-------	130	
	(*)	540	68	23	5	10	2.4	-------	-------	
	(*)	511	68	22	7	10	2.6	-------	-------	
	(*)	795	72	16	3	12	2.6	-------	-------	
5656-24:										
1927	10.1	1,022	95	26	4	11	2.2	6	183	Absent.
	11.4	958	90	22	4.5	10	2.1	6	154	Do.
	11.4	1,134	100	30	9	9	1.7	7	169	Do.
	11.7	1,022	100	30	6	9	1.8	6	170	Do.
	12.0	795	85	22	4	10	2.2	6	170	
	12.2	1,079	90	21	5	11	2.1	6	150	
	12.2	795	93	25	6	11	2.1	7	240	
	12.2	795	93	23	6	11	2.3	8	166	
	12.4	1,134	100	25	4.5	9	1.8	5.5	170	
	12.6	680	75	20	3	9	2.3	4	158	
	12.6	907	88	25	5	8	1.8	8	183	

* Undetermined.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928*—Continued

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
5656-24—Contd.	*Per cent*	*Grams*	*Mm.*	*Mm.*	*Mm.*	*Number*		*Mm.*	*Number*	
1927	12.6	1,079	85	23	6	10	2.3	7	230	
	12.6	852	93	23	5	8	1.8	6	200	
	12.7	907	88	18	4	12	2.6	7	205	
	12.8	907	90	23	6	10	2.1	7	276	
	12.9	568	85	22	4.5	11	2.7	4	175	
	13.0	795	85	23	7.5	10	2.2	5	175	
	13.0	738	75	18	3	9	2.2	4	190	
	13.1	958	93	24	5	10	2.1	8	180	
	13.3	1,022	105	25	4	10	2.0	5	211	
	13.5	680	78	22	6.5	10	2.1	4	189	
	13.5	625	73	17	4	10	2.8	6	142	
	14.4	795	83	23	5	8	2.0	6	158	
	14.6	738	83	20	4.5	11	2.5	6	182	
	14.9	511	75	20	3	11	2.7	5	210	
1928	13.2	568	80	24	7	10	2.1	6	140	
	14.3	795	90	20	4	10	2.0	7	157	
	15.2	1,164	95	26	7	10	1.9	7	116	
	15.4	1,134	100	22	4.5	9	1.6	8	160	
	15.6	937	98	27	6.5	9	1.8	7	167	
	16.2	795	83	24	4.5	9	2.2	6.5	110	
	16.2	937	93	22	4.5	11	2.0	6	113	
	16.6	540	77	20	5	9	2.0	5	130	
	16.6	852	90	18	5	11	2.1	6	103	
	17.0	1,022	82	23	5	10	1.9	6	116	
	17.0	710	70	19	4	10	2.3	4	105	
	17.0	568	75	23	7	10	2.3	5	130	
	17.4	882	86	24	5.5	10	2.1	6	140	
	17.5	768	92	22	4	11	2.2	6	150	
	17.8	710	95	25	6	10	2.2	7	116	
	18.2	825	90	20	4	12	2.2	5	98	
	18.2	568	73	24	7	9	2.1	6	193	
	18.3	710	77	22	4	10	2.1	7	133	
	19.0	1,134	97	18	3	11	1.9	5	116	
	19.9	598	77	18	3.5	10	2.4	6	175	
	(a)	312	65	14	3	10	2.7	5	143	
	(a)	568	75	16	2.5	12	2.6	4	123	
	(a)	483	82	18	3	10	2.2	6	153	
	(a)	453	70	18	4	9	2.1	5	123	
	(a)	453	67	15	4	12	3.0	5	127	
5942-24:										
1927	11.3	1,022	95	25	7.5	11	2.1	7	200	Present.
	11.3	680	85	27	7	9	2.1	8	80	Absent.
	11.5	680	75	21	4.5	11	3.2	7	160	Present.
	12.0	1,022	90	28	7	10	2.3	7	180	Do.
	12.4	680	80	23	4.5	11	2.6	7	75	Do.
	12.4	680	80	22	4.5	9	2.3		180	Do.
	12.6	625	80	20	4	10	2.6	6	123	Absent.
	12.7	1,250	95	25	5.5	13	2.5	8	150	Present.
	13.1	1,250	105	24	4.5	11	2.0	7	143	Do.
	13.2	958	90	25	5.5	9	2.1	6	193	
	13.3	738	85	22	4.5	11	2.4	6	170	Do.
	13.4	1,590	105	27	5	11	2.1	8	100	Do.
	13.6	738	85	26	5	10	2.5	7	159	Do.
	13.6	1,022	90	25	6	11	2.4	6	125	Absent.
	13.6	568	80	21	3.5	10	2.4	8	115	Do.
	13.8	852	95	23	5	11	2.3	7	145	Do.
	13.8	853	93	22	4	11	2.4	7	200	Present.
	14.1	625	80	20	3.5	11	2.7	7	121	Do.
	14.9	1,134	100	22	8.5	11	2.3	8	140	Do.
	15.0	568	80	18	3.5	11	2.8	4	108	Absent.
	15.0	795	90	25	3.5	10	2.1	7	140	
	15.1	795	95	23	5	13	2.9	5	110	Do.
	15.1	907	95	16				5		Do
	15.2	907	80	18	4.5	10	2.5	4	130	Do.
	15.8	625	83	20	5	11	2.6	6	120	Do.
1928	15.1	680	85	15	3.5	11	2.29	7	94	
	15.4	1,052	100	27	8	10	1.82	7	83	
	15.6	738	85	19	5	11	2.24	6	140	
	16.5	1,134	105	21	6	12	1.90	8	140	
	16.6	625	80	20	4	10	2.04	6	110	
	16.6	568	76	15	2	13	2.32	7	99	

[a] Undetermined.

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928*—Continued

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
5942-24—Contd.	*Per cent*	*Grams*	*Mm.*	*Mm.*	*Mm.*	*Number*		*Mm.*	*Number*	
1928	16.6	710	80	20	3.5	10	2.17	5.5	120	
	16.8	795	92	18	3	11	2.20	5	120	Absent.
	17.0	680	84	16	3	12	2.50	4.5	200	
	17.4	795	85	20	3.5	12	2.40	4	130	
	17.4	652	82	22	5.5	10	2.30	5.5	122	Do.
	17.5	765	85	17	1.5	12	2.45	5	83	
	17.8	907	93	23	5.5	11	2.10	8	150	Do
	17.9	820	90	20	3	13	2.29	6	99	
	18.0	568	80	21	2.5	11	2.75	5	84	
	18.2	985	100	17	3	12	2.32	8	100	
	18.4	795	87	18	2.5	12	2.40	5	160	Do.
	18.5	765	85	20	4.5	12	2.53	5.5	90	
	18.6	680	82	17	4	11	2.20	5.5	170	
	18.6	1,022	95	19	3	13	2.24	5	106	Do.
	18.7	1,022	97	22	3.5	11	2.10	8	190	
	19.0	907	98	13	1.5	12	2.18	6	100	
	19.0	540	76	14	2	13	2.60	6	105	
	19.0	680	82	15	3	13	2.95	5	72	
	19.8	595	78	22	6	11	2.20	4	72	
7794-24:										
1927	8.2	738	88	27	8	9	2.0	6	161	Present.
	8.6	907	95	30	7	11	2.3	6	172	Absent.
	8.9	738	80	19	4	10	2.5	5	120	Do.
	9.1	907	98	24	5	10	2.0	6.5	166	Present.
	9.8	738	93	22	4.5	11	2.3	6	90	Absent.
	10.1	958	90	21	8	9	2.1	6	175	Do.
	10.2	1,134	110	22	6	12	2.1	6	227	Present.
	10.2	680	78	18	3	11	2.8	3	200	Absent.
	10.4	852	85	16	4	12	2.2	4	154	Present.
	10.6	738	83	18	3	12	2.7	4	127	Do.
	10.7	1,022	85	24	3	11	2.1	6	160	Do.
	11.1	738	78	20	6	13	3.3	4	136	Absent.
	11.2	1,420	108	29	8.5	12	2.1	8	163	Present.
	11.9	738	83	25	5	8	2.0	6	208	Do.
	12.0	1,022	95	21	8	12	2.3	6	145	Do.
	12.0	738	90	22	4	10	2.2	5	166	Do.
	12.3	958	88	22	5	10	2.2	5	166	Do.
	12.3	738	78	21	3.5	8	2.0	5	200	Absent.
	12.4	1,022	85	22	3	9	2.1	4	166	Present.
	12.7	958	95	23	5	12	2.3	5	200	Do.
	12.8	1,250	108	30	11	11	2.0	7	154	Absent.
	12.9	625	74	21	5	11	3.0	6	122	Do.
	13.2	852	85	28	9	12	3.0	7	140	Do.
	13.2	958	73	22	4	10	2.4	5	172	Do.
	13.3	568	73	18	4	15	3.5	4	200	Present.
1928	13.2	765	85	15	2	12	2.26	6.5	144	
	14.2	738	84	18	2.5	9	2.25	6.5	123	
	14.2	738	92	18	3	10	2.09	8	113	
	14.4	795	95	20	4	10	2.13	7	144	
	14.4	650	85	17	2	10	2.44	4	143	
	14.6	710	83	21	4.5	11	2.75	6	146	
	14.7	1,050	88	21	5	13	2.32	6.5	147	
	15.0	907	85	20	5	11	2.29	6	143	
	15.0	765	80	20	3.5	11	2.44	5.5	130	
	16.0	825	92	17	2.5	10	2.00	7	142	
	16.0	765	86	17	4	11	2.24	7	132	
	16.2	907	90	20	4	13	2.45	7	143	
	16.4	907	75	18	2.5	11	2.55	5	149	
	16.5	1,022	95	22	6	11	2.20	6.5	92	
	16.6	907	95	17	2	9	1.76	6.5	140	
	16.7	650	75	17	3	11	2.62	5	130	
	17.2	1,022	100	18	3.5	12	2.03	6	134	
	17.3	935	86	22	4	12	2.40	6	131	
	17.3	480	78	19	3.5	10	2.27	4	138	
	17.6	680	83	20	3	10	2.32	6	110	
	17.8	625	75	18	4	11	2.55	4	92	
	18.6	568	68	15	2.5	11	2.44	4.5	145	Absent.
	18.8	907	82	21	6	12	2.29	-------	94	
	19.5	595	74	17	3	13	2.88	5	107	

TABLE 2.—*Macroscopic, chemical, and microscopic data on mature beets grown at Fort Collins, Colo., in 1927 and 1928*—Continued

Selection No. and year tested	Sugar	Weight	Diameter			Rings	Ring density coefficient	Microscopic data on fourth ring		
			Total	First ring	Central core			Width of vascular zone	Vascular bundles per decimeter	Lignified sheath cells
8753–24:	*Per cent*	*Grams*	*Mm.*	*Mm.*	*Mm.*	*Number*		*Mm.*	*Number*	
1927	11.3	680	88	24	5	10	2.2	6	125	Absent.
	12.1	680	98	26	5	9	1.7	8	191	Do.
	12.1	1,134	98	25	5.5	9	1.9	8	163	Do.
	12.7	625	75	26	7	9	2.4	5	146	Present.
	12.7	1,306	103	21	3	8	1.5	7	138	Absent.
	12.9	1,134	105	30	8.5	10	2.0	6.5	123	Do.
	13.3	852	85	20				5.5	140	Do.
	13.5	1,022	100	20	4	13	2.3	6	164	Do.
	13.6	852		23	4	10	2.1	6.5	177	Present.
	13.6	795	88	25	6	8	1.7	6.5	161	Do.
	13.7	1,079	90	28	6	8	1.8	8	166	Do.
	13.9	795	93	21	6	10	2.0	6.5	170	Absent.
	14.0	852	92	25				7	148	Do.
	14.1	852	93	23	5.5	10	2.0	7	100	Present.
	14.2	795	88	25	5.5	9	2.0	5	142	Absent.
	14.3	1,022	90	20	6	8	1.6	6	158	Do.
	14.3	738	80	27	11	9	2.3	6	142	Do.
	14.7	680	80	26	8	8	2.0	6.5	177	Do.
	14.7	680	90	25	4	8	2.0	4	125	Do.
	14.8	907	85	20	4	11	2.5	5	100	Present.
	15.0	628	80	22	4	9	2.0	7	175	Absent.
	15.1	680	83	16	4	9	2.3	4	138	Present.
	15.2	907	90	22	5	9	2.1	6	171	Do.
	15.2	1,134	103	19				7	160	Absent.
	16.7	738	88	20	5	11	2.6	4	191	Present.
1928	15.2	852	93	22	5	11	2.2		120	Absent.
	15.3	1,052	100	18	4	12	2.2		130	Do.
	15.4	680	85	25	4	9	1.9		160	
	15.8	795	92	21	5	10	1.9		160	
	16.2	958	99	30	9	10	1.9		80	Absent.
	16.2	907	95	25	5	10	2.0		130	
	16.4	1,022	95	25	5.5	11	2.2		130	Absent.
	16.6	738	81	18	5	9	2.0		230	
	16.8	988	100	23	5.5	10	1.8		120	
	17.0	825	95	17	3	10	2.0		140	Do.
	17.0	795	87	17	3	11	2.3		170	
	17.4	768	88	21	3	11	2.3		100	Do.
	17.6	710	92	22	5	9	1.8		130	
	17.6	1,022	90	15	3	10	2.1		130	
	17.7	795	89	24	5	11	2.0		110	Present.
	18.0	768	81	20	4	12	2.8		90	Absent.
	18.4	568	84	19	4	9	2.1		90	Do.
	18.5	795	90	25	4.5	10	1.9		140	
	19.1	768	85	19	4	10	2.1		120	
	19.2	710	87	20	4	10	2.1		120	Present.
	(*)	655	80	17	3	10	2.1			
	(*)	625	75	18	3	9	2.2			
	(*)	768	83	21	4	10	2.3			
	(*)	1,250	105	24	6	11	1.7			
	(*)	1,079	104	23	5	9	1.7			

TABLE 3.—*Mean (M), standard deviation (σ), and coefficient of variability (CV) of diameters of first ring and central core, of ring numbers, of ring-density coefficients, and of numbers of bundles per decimeter in the fourth ring of 30 sugar-beet selections*

[Selections listed in numerical order]

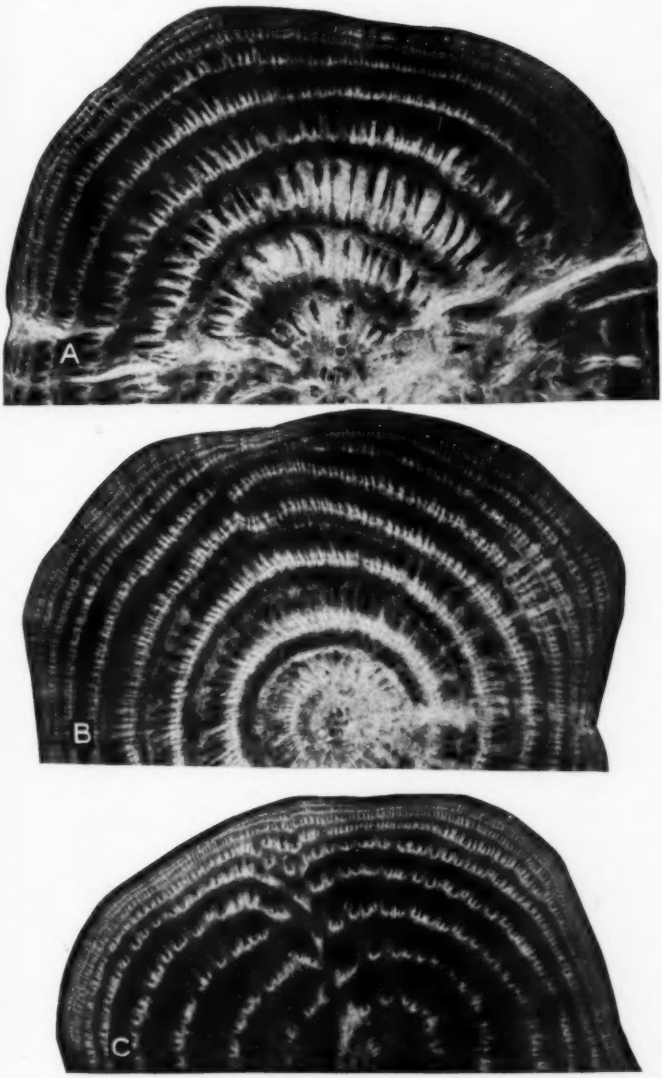
Selection No.	Grown		First ring			Central core			Number of rings			Ring-density coefficient			Number of bundles per decimeter of fourth ring		
	Locality	Year	M	σ	CV	M	σ	CV	M	σ	CV	M	σ	CV	M	σ	CV
			Mm.			*Mm.*											
150-24	Fort Collins	1927	23.4	3.58	15.30	5.7	1.69	29.65	10	1.27	12.70	2.25	0.247	10.98	156	28.3	18.14
	do	1928	20	2.86	14.30	3.6	1.01	28.06	10.7	1.22	11.40	2.18	.217	9.95	156	24.0	15.38
	Fort Lewis	1928	18.3	5.23	28.57	4.1	1.92	46.83	10	1.03	10.30	2.00	.579	28.95	139	29.3	21.08
193R-25	Fort Collins	1927	17.5	3.43	19.60	3.5	1.54	44.00	10.4	1.11	10.67	2.23	.256	11.48	166	41.9	25.24
554-24	do	1927	18	3.37	18.72	3.0	1.13	37.67	9.7	1.08	11.13	2.25	.373	16.58	174	28.8	16.55
	do	1928	15	2.31	15.40	2.5	1.04	41.60	10.8	1.37	12.68	2.48	.269	10.85	165	15.0	9.09
	Fort Lewis	1928	15	3.37	22.46	2.5	.78	31.20	10.8	1.27	11.76	2.50	.321	12.84	152	33.1	21.78
600s-25	Fort Collins	1927	22	3.70	16.82	5.3	2.07	39.06	10.4	1.44	13.85	2.19	.244	11.14	138	25.4	18.40
	do	1928	22	3.08	14.00	4.6	1.49	32.39	11.2	1.43	12.77	2.04	.227	11.13	137	27.3	19.93
	Fort Lewis	1928	19	3.79	19.95	4.3	.98	22.79	9.9	1.24	12.52	2.04	.211	10.34	119	16.0	13.44
730-24	Fort Collins	1927	19	3.83	20.16	3.3	1.55	46.96	10.1	1.00	9.90	2.38	.328	13.78	149	23.3	15.64
	do	1928	18.5	2.80	15.14	3.5	1.01	28.86	10.4	.98	9.42	2.12	.226	10.66	154	28.8	18.70
	Fort Lewis	1928	17	3.53	20.76	3.5	1.21	34.57	10.1	1.17	11.58	2.06	.271	13.16	147	40.8	27.75
898-24	Fort Collins	1927	21.6	3.47	16.06	5.5	2.10	38.18	10.4	.92	8.85	2.26	.199	8.80	136	25.4	18.68
	do	1928	19.6	2.61	13.32	4.6	1.18	25.65	11	.87	7.91	2.24	.222	9.91	125	19.2	15.36
	Fort Lewis	1928	17.3	3.17	18.32	4.4	1.33	30.23									
992s-25	Fort Collins	1927	20.3	2.97	14.63	4.7	1.39	29.57	10.6	1.77	16.70	2.30	.264	11.48	159	13.6	8.55
	do	1928	17.8	2.30	12.92	3.7	1.44	38.92	12.1	1.26	10.41	2.46	.209	8.50	153	20.1	13.14
	Fort Lewis	1928	14	3.32	23.71	2.4	1.13	47.08	12.1			2.40	.353	14.71	166	31.3	18.85
1161-24	Fort Collins	1927	20	2.43	12.15	4.8	1.47	30.62	9.7	1.08	11.13	2.13	.241	11.31	154	29.9	19.42
	do	1928	15	2.03	13.53	2.6	.60	23.08	9.7	.62	6.39	2.10	.177	8.43	146	17.7	12.12
	Fort Lewis	1928	15	2.48	16.53	3.2	.99	30.94				2.10	.237	11.29	147	19.3	13.13
1228s-25	Fort Collins	1927	23	2.09	9.09	4.7	1.08	22.98	9.4	.97	10.32	1.94	.191	9.84	149	22.6	15.17
	do	1928	19.5	3.30	16.92	3.5	1.02	29.14	9.7	.83	8.56	1.96	.191	9.74	120	30.9	25.75
	Fort Lewis	1928	19	2.62	13.79	4.8	2.86	59.58				2.00	.100	5.00	131	26.3	20.08
1293s-25	Fort Collins	1927	19	2.22	11.68	4.3	.96	22.33	10.5	1.12	10.67	2.41	.209	8.67	179	36.8	20.56
	do	1928	16.4	2.44	14.88	2.9	.83	28.62	10.8	1.39	12.87	2.37	.267	11.27	115	8.8	7.65
	Fort Lewis	1928	11.8	1.96	16.61	2.1	.95	45.23									
1427-24	Fort Collins	1927	21.6	3.47	16.06	5.5	2.23	40.54	10.4	.99	9.52	2.26	.199	8.80	155	34.0	21.93
	do	1928	17	3.29	19.35	3.0	1.22	40.67	10.4	1.12	10.77	2.49	.304	12.21	162	33.5	20.68
	Fort Lewis	1928	18	4.57	25.39	3.6	2.90	80.56	10.5	1.20	11.43	2.30	.414	18.00	163	28.5	17.48
1569-24	Fort Collins	1927	22	2.80	12.72	6.0	1.56	26.00	9.7	.62	6.39	2.23	.253	11.35	144	32.8	22.78
	do	1928	21	2.49	11.86	4.4	1.29	29.32	10.9	.63	5.78	2.17	.155	7.14	136	26.6	19.56
	Fort Lewis	1928	17	3.15	18.53	3.4	1.21	35.59				2.40	.217	9.04	125	20.1	16.08
1591-24	Fort Collins	1927	23.7	3.10	13.08	5.6	1.13	20.18	10	1.44	14.40	2.15	.246	11.44	140	15.8	11.28
1612-24	do	1927	20.4	2.73	13.38	4.7	1.64	34.89	10.5	1.02	9.71	2.26	.213	9.42	146	34.4	23.56
	do	1928	18.5	2.99	16.16	3.2	1.15	35.94	10.9	.89	8.17	2.20	.197	8.95	153	24.7	16.14
	Fort Lewis	1928	19	4.14	21.79	4.3	1.12	26.05				2.20	.589	26.77	133	30.6	23.01

TABLE 3.—*Mean (M), standard deviation (σ), and coefficient of variability (CV) of diameters of first ring and central core, of ring numbers, of ring-density coefficients, and of numbers of bundles per decimeter in the fourth ring of 30 sugar-beet selections*—Continued

Selection No.	Grown		First ring			Central core			Number of rings			Ring-density coefficient			Number of bundles per decimeter of fourth ring		
	Locality	Year	M	σ	CV	M	σ	CV	M	σ	CV	M	σ	CV	M	σ	CV
			Mm.			*Mm.*											
18188–25	Fort Collins	1927	21	3.30	15.71	4.3	1.41	32.79	10.5	1.10	10.47	2.24	.390	17.41	146	30.4	20.82
	do	1928	19	3.12	16.42	2.9	.76	26.21	11.8	.98	8.31	2.27	.279	12.29	155	29.8	19.23
	Fort Lewis	1928	14	4.86	34.70	2.3	1.01	43.91									
18458–25	Fort Collins	1927	18.1	3.23	17.84	3.1	.94	30.32	10.4	1.25	12.02	2.31	.266	11.52	121	23.5	19.42
2287–24	do	1927	26	4.44	17.08	6.3	2.21	35.07	9.3	1.01	10.86	2.10	.234	11.14	155	21.7	14.00
2310s–25	do	1927	22	3.86	17.54	5.4	1.93	35.74	10.8	1.56	14.44	2.28	.274	12.02	162	29.8	18.40
2346s–25	do	1927	25	3.81	15.24	5.7	2.12	37.19	11.3	1.01	8.94	2.23	.190	8.52	118	29.4	24.91
2445s–25	do	1927	21	4.17	19.86	3.8	1.65	43.42	10.4	1.33	12.79	2.50	.388	15.52	184	30.9	16.79
3403–24	do	1927	22	3.73	16.95	5.8	2.01	34.66	10	1.10	11.00	2.22	.242	10.90	152	27.1	17.83
	do	1928	17	3.56	20.94	3.2	1.43	44.69	10.7	1.01	9.44	2.40	.326	13.58	126	19.6	15.55
	Fort Lewis	1928	16.8	2.71	16.13	3.2	1.23	38.43	9.6	.60	6.25	2.43	.319	13.13	159	17.6	11.07
3956–24	Fort Collins	1927	18.5	3.42	18.49	4.3	1.35	31.40	12.3	1.64	13.33	2.48	.337	13.59	126	23.8	18.89
	do	1928	16	2.87	17.94	3.1	.95	30.65	14.2	1.58	11.13	2.70	.353	13.07	97	20.6	21.24
	Fort Lewis	1928	14.7	2.44	16.60	2.7	.81	30.00				2.70	.515	19.07	109	16.4	15.05
4545–24	Fort Collins	1927	20	3.06	15.30	4.9	1.82	37.14	11	1.34	12.18	2.70	.353	13.07	175	28.5	16.28
	do	1928	20	2.97	14.85	3.7	1.37	37.03	11	1.40	12.73	2.60	.249	9.58	140	17.9	12.79
	Fort Lewis	1928	18.5	2.70	14.59	3.4	1.50	44.12									
4621–22	Fort Collins	1927	25	4.49	17.96	6.6	2.14	32.42	10	1.18	11.80	2.23	.312	13.99	191	13.5	7.07
5656–24	do	1927	23	3.13	13.61	5.0	1.38	27.60	10	1.04	10.40	2.18	.310	14.22	185	28.5	15.41
	do	1928	21	3.39	16.14	4.7	1.35	28.72	10.2	.92	9.02	2.16	.286	13.24	133	29.7	22.33
	Fort Lewis	1928	19	4.70	24.73	4.1	1.78	43.41				2.17	.281	12.95	120	24.1	20.08
5942–24	Fort Collins	1927	23	3.00	13.04	5.0	1.38	27.60	10.6	.98	9.25	2.42	.284	11.74	140	34.4	24.57
	do	1928	19	3.13	16.47	3.7	1.54	41.62	11.6	.99	8.53	2.30	.242	10.52	118	34.3	29.07
	Fort Lewis	1928	18	2.73	15.16	4.0	1.12	28.00									
6077–24	Fort Collins	1927	20	3.95	19.75	3.6	2.29	63.61	9.2	.97	10.54	2.24	.329	14.69	200	27.9	13.95
	do	1928	15	2.56	17.06	2.0	.78	39.00	10.4	1.26	12.11	2.40	.326	13.58	119	23.4	19.66
	Fort Lewis	1928	13.5	2.63	19.48	1.7	.43	25.29				2.27	.393	17.31	163	33.6	20.61
7794–24	Fort Collins	1927	23	3.75	16.30	5.5	2.11	38.36	10.8	1.56	14.44	2.38	.377	15.84	164	30.9	18.84
	do	1928	19	2.00	10.53	3.5	1.16	33.14	10	1.19	11.90	2.23	.250	11.21	130	18.0	13.85
	Fort Lewis	1928	19	5.19	27.31	3.8	2.30	60.52				2.32	.361	15.56	154	23.3	15.13
8146–24	Fort Collins	1927	21	4.18	19.90	3.7	1.30	35.13	9.6	1.11	11.56	2.20	.398	18.09	144	14.3	9.93
	do	1928	16.6	2.55	15.36	2.9	.68	23.45	10.7	1.10	10.28	2.15	.266	12.37	118	24.8	21.02
	Fort Lewis	1928	15	3.18	21.20	2.3	.77	33.48									
8753–24	Fort Collins	1927	23	3.07	13.35	5.5	1.78	32.36	9	1.26	14.00	2.04	.277	13.58	152	24.2	15.92
	do	1928	21	3.26	15.52	4.5	2.98	66.22	10	.89	8.95	2.06	.231	11.21	130	32.4	24.92
	Fort Lewis	1928	14.5	2.99	20.62	2.4	.82	34.17	9.7	.73	7.52	2.56	.385	15.04	152	29.4	19.34

Cross sections of sugar-beet selections. Natural size: A, No. 4621–22 with 14.6 per cent sugar; B, No. 2346s–25 with 14.2 per cent sugar

Cross sections of beets of selection No. 1293s-25. Natural size: A, 16 per cent sugar; B, 13.1 per cent sugar



Cross section of three sugar beets of selection No. 1845s–25. Natural size: A, 12.5 per cent sugar; B, 15.8 per cent sugar; C, 12.9 per cent sugar

METEOROLOGICAL DATA

The meteorological data for the growing seasons of 1927 and 1928 are given in Table 4. These data show that as compared with the season of 1928 the 1927 season was characterized by a greater rainfall, slightly higher temperature, and higher relative humidity.

TABLE 4.—*Meteorological data for April to October of 1927 and 1928 at Fort Collins, Colo., with normal data for comparison*

[Furnished by the Colorado Agricultural Experiment Station]

Year and month	Temperature					Relative humidity		Precipitation
	Maximum	Mean maximum	Mean	Minimum	Mean minimum	7 a. m.	7 p. m.	
	° F.	° F.	° F.	° F.	° F.	Per cent	Per cent	Inches
1927:								
April	81.5	58.6	45.2	17.0	31.7	72.2	53.9	2.69
May	86.0	71.5	56.3	30.0	41.1	56.0	40.4	.91
June	93.0	74.8	61.9	38.9	49.1	76.8	62.9	2.17
July	91.0	81.6	67.6	47.6	53.6	70.2	56.0	2.19
August	84.8	76.4	64.1	45.0	51.8	81.0	68.4	2.10
September	85.2	72.7	58.9	28.0	45.0	73.6	63.4	1.10
October	80.5	66.7	50.2	25.0	33.6	75.4	54.6	1.05
Average	86.0	71.8	57.7	33.1	43.7	72.2	57.1	
Total								12.21
1928:								
April	75.6	57.8	43.3	8.8	28.9	63.0	42.2	.98
May	87.1	68.4	55.7	31.1	43.0	70.2	64.9	3.35
June	83.0	68.9	57.3	35.8	45.7	75.8	62.8	2.73
July	91.0	82.2	67.9	44.0	53.6	69.0	59.3	.83
August	94.7	82.0	66.2	42.0	50.4	64.7	50.5	.69
September	87.2	74.7	58.5	31.8	42.3	65.7	49.0	.09
October	81.0	60.0	47.0	22.0	34.0	77.6	63.8	1.50
Average	85.7	70.6	56.6	30.8	42.6	69.4	56.1	
Total								10.17
Normal:								
April		59.7	45.5		31.5	68.3	52.3	2.09
May		67.8	54.1		40.4	69.0	56.6	2.85
June		78.1	63.3		48.2	69.5	57.9	1.62
July		83.3	68.3		53.4	71.4	61.2	1.80
August		83.0	67.2		51.7	72.7	60.9	1.18
September		75.4	59.1		43.0	74.1	60.8	1.26
October		63.8	47.6		32.2	74.8	59.5	1.17
Average		73.0	57.9		42.9	71.4	58.5	
Total								11.97

GENERAL APPEARANCE AND COLOR OF FLESH

Freshly cut horizontal sections of mature beets show a peculiar zonation produced by concentric rings of alternating vascular tissue and parenchyma with the sharpest line of demarcation in the region of the cambium. (Pls. 1, 2, and 3.) The distinctness of this zonation varies greatly in different beets and appears to be bound up with color differences of the flesh. Our cultivated sugar beets have a whitish or ivory-colored flesh which, in poor types, is watery greenish or yellow in hue. Beets with a pure-white flesh usually show the least marked zonation, since the vascular tissue merges imperceptibly with the interzonal parenchyma. Such beets give the impression of having very narrow vascular zones separated by broad bands of parenchyma. However, when thin sections of these beets are viewed

against a black background, the width of the vascular tissue will often prove to be much greater than it appeared to be at first glance. Pseudonarrow vascular zones are occasionally found in beets with an ivory-white flesh, but the phloem usually contrasts strongly with the adjacent parenchyma and only the xylem merges imperceptibly into the surrounding tissue. (Pl. 3, C.) Occasionally both phloem and xylem are indistinct, whereas the cambium region stands out as a sharply delimited white or yellowish line. In general, however, color differences suffice to set off the vascular tissue from the surrounding parenchyma. Contrary to general belief, the parenchyma tissue is commonly restricted to a very narrow concentric zone. Within the vascular ring the individual bundles, because of their dark color, contrast strongly with the adjacent parenchyma (pl. 2, B), while xylem and phloem are separated by the white line previously mentioned. It is because of this contrasting coloration of the cambium that what appear as rings when casually observed are in reality made up of the elements of two adjacent rings with a band of interzonal parenchyma in the middle. Flesh color and ring prominence may be correlated with percentage of sucrose, while very broad and prominent vascular rings are typical of certain selections and aid in their characterization.

RING NUMBER AND DENSITY

The rings or annular zones of growth referred to above are more or less equidistant except near the periphery, where they are very close together. The number of the mature rings more or less widely spaced is about five, and this comprises approximately one-half of the total number of rings formed.

Schindler and Proskowetz (*10*) counted the rings in Klein Wanzleben, Ameliorée, and Vilmorin Rose, and found that Ameliorée had a consistently smaller number of rings than the other two varieties. Ring counts on beets were also reported by Janasz (*6, p. 951*), who, however, came to the conclusion that beets behave as individuals, each with its own characteristic ring number.

The data reported in this paper show that in some selections, for example in No. 3956–24 (Table 3), the ring number is consistently and conspicuously high, whereas in others, notably Nos. 1228s–25 and 2287–24 (Table 3), it is characteristically low. There was a considerable amount of variation in the number of total and mature rings in the selections studied, but in general the variation appeared to be less in individuals of a given selection. A high total ring number is frequently correlated with a high mature number, i. e., in a beet with 12 rings 7 may be mature, but occasionally beets with a high mature number have a comparatively low total. Individual beets of the latter type were found in all selections.

Ring determinations on beets of different weight and diameter fail to show definite relationships. Beets of large diameter will occasionally give a high ring count, but so will small-sized beets. In this respect the different selections behave alike. Kraus (*7*) made similar observations on Klein Wanzleben and Imperial varieties. He found that a decrease in the diameter of the beet is not followed by a decrease in the number of rings, and that beets of the same variety which have an equal diameter may have, nevertheless, a different number of rings.

The width of the three inner rings varies but little; the fourth ring is usually narrower, and the fifth appreciably so. In selections with a small central core the width of the first ring is usually less than that of the two succeeding rings. Table 5 gives individual ring measurements for three beet selections.[4] The beets are all arranged in a series. Those of the first group (selection No. 554–24) are arranged from low to high percentage of sucrose, those of the second group (selection No. 150–24) from low to high weight, and those of the third group (selection No. 7794–24) from small to large diameter, as measured in the neck region. Ring width appears in many cases to be correlated with weight, and there is a slight relationship between ring width and ring number. A negative correlation is frequently observed in beets with a low ring number; i. e., beets with only 8 rings will have no wider rings than beets with 10 or 12 rings. This condition is probably due to the unbalanced ratio between mature and peripheral rings, caused by a reduction in the number of peripheral but not mature rings. Occasionally, however, the number of mature rings is also reduced, and in such instances the rings are very wide. Beets of this type were frequently observed in selection No. 2287–24 (Table 1). Of more frequent occurrence are selections with relatively narrow rings. In such beets the number of mature rings is 6 and frequently even 7.

TABLE 5.—*Individual ring measurements in three mature beet selections grown at Fort Collins, Colo., 1927*

Item	Core width	Mature ring widths					Immature rings	
		No. 1	No. 2	No. 3	No. 4	No. 5	Number	Width
Selection No. 554–24 (arranged in series from low to high sugar content)	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*		*Mm.*
	2.5	7	6	6	5	4	5	4.5
	2	5	7	7	7	3	4	3.5
	1.5	8	7	6	5	3	6	6.5
	2	8	8	7	7	4	5	8
	1.5	5	6	5	6	5	5	10.5
	6	7	7	7	7	4	5	5.5
	4	8	10	7	7	3	3	3
	3	6	6	6	5	5	6	7
	1.5	5	8	9	8	7	5	7.5
	2.5	8	8	8	7	4	6	4
	3	7	9	6	5	4	7	8.5
	3	7	10	8	7	6	4	6
	4	7.5	10	10	8	5	5	5
	2.5	7.5	9	9	7	3	4	4
	2.5	10	10	6	6	5	3	5
	1.5	6	8	8	7	6	5	11
	2.5	8	9	8	7	6	4	6.5
	4.5	8	10	8	6	3	4	5
	3	7	8	9	8	6	5	9
	3	9	10	9	8	5	3	4.5
	4	7	8	8	7	4	4	3.5
	3.5	5	6	14	4	6	3	8
	4	9	8	8	7	6	3	12
	5	10	10	10	6	6	6	10
	2.5	9	10	10	8	6	5	7.5
Mean	3.0	7.3	8.3	8.0	6.6	4.8	4.6	6.6
Standard deviation	1.14	1.42	1.4	1.85	1.04	1.21	1.1	2.48
Coefficient of variability	38.20	19.45	16.87	23.12	15.76	25.21	23.91	37.6

[4] Originally 9 groupings were made, but since the 3 selections behaved more or less alike, 3 groups were selected and condensed into 1 table. Additional data for each are given in Table 1.

TABLE 5.—*Individual ring measurements in three mature beet selections grown at Fort Collins, Colo., 1927*—Continued

Item	Core width	Mature ring widths					Immature rings	
		No. 1	No. 2	No. 3	No. 4	No. 5	Number	Width
Selection No. 150–24 (arranged in series from low to high weight)	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*		*Mm.*
	3.5	10	8	8	5	4	4	4
	4	10	10	8	5	3	4	5
	4.5	10	8.5	7	6	3	4	4
	7.5	13	11	8	4	2	4	2
	5	10	8	7	6	4	6	6
	6	12	9	7	6	3	4	4
	2.5	9	8	8	6	5	8	12
	5	10	8	9	7	4	4	5
	5.5	11	9	8	6	4	6	8
	6	11	8	7	6	4	6	4
	7.5	14	9.5	7.5	6	4	5	7
	5	12	10	8	5	3	5	4
	5	11	8	8	6	3.5	6	7.5
	5.5	10	8	8	7	5	7	10
	5.5	13	9	7.5	7	5.5	5	8.5
	7	14	11	9	7	5	4	5
	6	11	9	9	7	6	5	6.5
	5.5	10	8.5	8.5	7	5	4	5
	6.5	12	9.5	7.5	5	2	3	2.5
	9	15	10	8	6	4	5	6
	3.5	10	10	9	7	6	6	6
	5.5	11	9	8	7	6	8	12
	7	13	11	9	8	5	5	5
	4	11	10	10	7	4.5	5	4.5
	9	16	9	10	7	6	6	8
Mean	5.6	11.6	9.2	8.2	6.2	4.3	5.2	6.1
Standard deviation	1.69	1.65	.99	.84	.91	1.17	1.26	2.53
Coefficient of variability	30.18	14.22	10.76	10.21	14.67	27.21	24.23	41.47
Selection No. 7794–24 (arranged in series from small to large neck diameter)								
	8	13	10	8	6	4	4	5
	6	15	10	8	5	4	6	4
	3	9	8	7	6	5	5	5
	5	12	9	9	9	6	5	6
	4	11	10	8	7	5	6	8
	7	10	9	8	5	5	4	6
	3	10	7	6	5	3	6	7
	5	11	8	9	8	7	7	15
	4	8	8	8	7	5	7	6
	3	8	9	7	5	5	7	10
	4	12	9	9	8	6	6	8
	6	10	8	6	6	5	8	10
	8	15	12	10	9	6	7	7
	5	12	10	8	6	5	4	2
	5	11	10	8	6	4	5	6
	7	11	9	9	7	6	7	9
	4	10	8	8	6	3	4	4
	4	11	10	8	6	4	5	7
	3	11	10	8	6	4	4	4
	5	11	9	8	8	5	7	10
	11	15	9	10	7	6	6	8
	5	11	7	6	5	3	6	5
	4	11	8	5	5	3	5	5
	10	14	9	8	6	4	8	6
	4	9	5	5	5	3	10	4
Mean	5.3	11.2	8.8	7.8	6.4	4.6	6.0	6.7
Standard deviation	2.11	1.89	1.35	1.31	1.23	1.16	1.48	2.67
Coefficient of variability	39.81	16.87	15.34	16.79	19.27	25.22	24.67	39.85

Since ring width and ring number are readily measured characters, they attracted the attention of earlier investigators. Bolsunow (*2*) studied the structure of a collection of 109 sugar-beet varieties and noticed that in the Asiatic varieties the rings were uniformly narrower than in the American varieties. Vivien (*13*) observed that rich beets had narrower rings and a larger number of them than poor beets, and before him Hoffmann (*5*) recorded observations showing that

within certain limits the width of the rings varied independently of the sugar content and that in especially rich beets the width of the mature rings varied between 3 and 6 mm.

While it is undoubtedly true that medium or small beets with a conspicuously large number of rings are rich in sugar, it is equally true that beets with an average number of 9 to 11 rings may be either rich or poor. In such cases, however, other characters which will be described later may aid in segregating the poor from the rich forms.

While working on the development of the different tissues of the beet, Seeliger (*12*) also made observations on the relationship between ring density and sugar content. He defined ring density as the quotient obtained by dividing the total number of rings by the radius of the beet measured in centimeters. He gave the ring density for beets of different ages and sizes and also noted that the ring density did not change after the beet reached a radius of 2.8 cm. Seeliger suggested the use of ring-density measurements as a supplementary selection method in beet-breeding work.

More extensive data on ring density were published by Pack (*8*). After analyzing the measurements on 500 beets he concluded that there exists a small positive correlation between ring density and percentage of sucrose and a negative correlation between ring density, weight, and total sucrose.

The ring-density coefficient should be a more accurate index of percentage of sucrose than either ring number or ring width, since it takes cognizance of both these variables. Unfortunately, ring number and ring width show only a general and inconsistent relationship to percentage of sucrose, which explains why rich beets occasionally have a low ring-density coefficient and poor beets an abnormally high one. In general, it may be assumed that a high ring-density coefficient is indicative of high sugar trend, and if polariscopic analysis fails to verify this conjecture one must look for other characters, viz, color of the flesh and composition of the vascular tissue, to explain the contradiction.

THE FIRST OR INNERMOST RING

Since the mature rings are more or less evenly spaced, differences in width, unless actually measured, are not readily noticed. Width differences of the first ring, however, easily catch the eye, since the ring is centrally located and can be viewed in its entirety without confusion with other rings. For example, in comparing the measurements of beets from the two selections Nos. 2287–24 and 554–24 (Table 1), the fairly consistent ring-width difference becomes noticeable at once, permitting fairly complete separation of these selections by this character alone.

Selections which are characterized by a large first ring have commonly fewer rings than selections in which the first ring is small or of medium size. That this does not always hold true can be seen by comparing selections Nos. 8753–24 and 7794–24. (Table 3.) Although the ring size is the same in both selections, the average ring number is only 9 for the first and 10.8 for the second group. Individual variations within a selection are common, but they can most often be associated with differences in the size of the beets. There is a tendency for large-sized beets of a selection to have a larger first ring

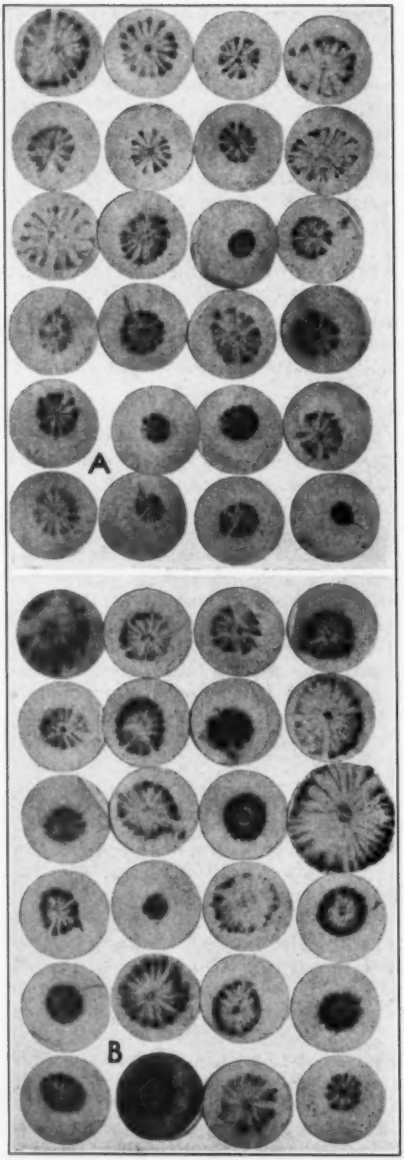


FIGURE 1.—Cross section from central cores of sugar beets, arranged according to weight from left to right with first section from lightest beet. ×1½. A, Selection No. 150-24; B, selection No. 2287-24

than small or medium-sized individuals, though occasionally the opposite is true. In selections with a typical small-sized first ring the influence of differences in beet size and ring width is not so noticeable, and very large individuals with a very small first ring are frequently seen.

Definite relationships between the size of the first ring and the percentage of sucrose can not be determined unless the difference in number of rings is taken into consideration; but although it would be expected, a priori, that of two beets possessing a large first ring the one having the larger total ring number would be richer in sugar than the one having fewer rings, the measurements recorded in Tables 1 and 2 failed in most cases to confirm this assumption.

THE CENTRAL CORE

The central core forms a 2-lobed, more or less star-shaped structure of which the innermost part constitutes the primary xylem plate. It is most uniform in the neck region, its symmetry being later broken by the passing out of radial strands of vascular tissue—the root traces. Serial sections through a beet from the neck region to the taproot end show that the size of the central core varies somewhat, being usually smaller in the taproot end. Occasionally the central core will be considerably wider in the neck region, which is especially true of broad, rapidly tapering beets. To avoid injury to the beets Hoffmann (*5*) examined the central core at the taproot end primarily.

He found that the anatomical structure of the central core in this region is very symmetrical and well suited for comparative studies.

The data obtained in the present studies indicate that the measurements of the central core are of practical value in the anatomical characterization of the selections, since size differences of the core are easily determined and compared. The majority of the selections studied have a medium-sized core, but certain selections stand out preeminently because of their very large or very small cores, as can be seen from Figures 1, 2, and 3. The degree of uniformity of core size differed in the selections studied. In some, notably those having a very small core, the uniformity is striking (fig. 3, B), and the same is true, though to a lesser degree, of some of the large-core types. On the other hand, there are some selections which at present can not be placed in any group, since the variation in core size is too marked. The size of the central core is slightly influenced by the diameter of the first ring. In selection No. 2287–24 (Table 2), where the individuals have a uniformly large first ring, the central core is also large. Likewise in selections No. 554–24 (Table 2), where the first ring is small, the core is also greatly reduced. Judging from the material used, the size of the central core and the weight of the beet do not seem to be related to each other, and there appears to be no relation between the size of the core and the percentage of sucrose. These facts are evident from an examination of Figures 1, 2, and 3, in which the individual photographs are arranged in an ascending series from low to high percentage of sucrose. In short, the size of the central core is a definite character apparently characteristic of a type, and the indications are that it will form a valuable morphological character for type description rather than for sucrose percentage.

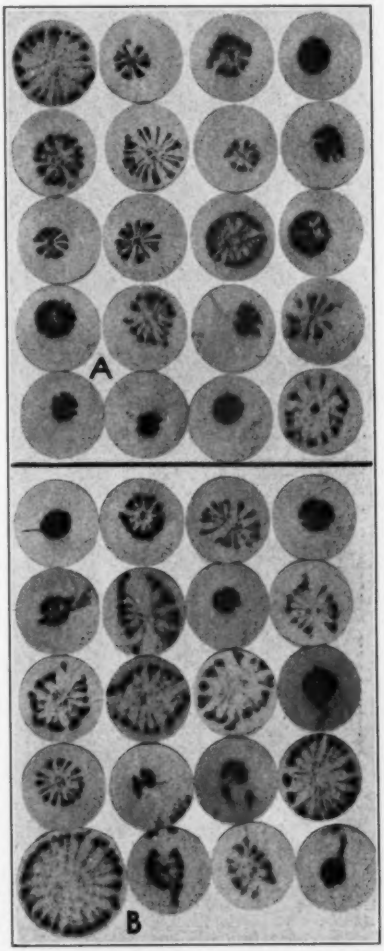


FIGURE 2.—Cross sections from central cores of sugar beets, arranged according to weight from left to right with first section from highest beet. × 1½. A, Selection No. 1612–24; B, selection No. 1427–24

The vascular tissue of the central core is composed of numerous bundles which converge toward the central xylem plate. In large cores these bundles or rays terminate some distance from the center, and only a few scattered elements extend farther in. The number of core rays is somewhat larger at the taproot end of the beet than in the neck region (Table 6), although the actual size of the core is commonly smaller at the taproot end. The size and number of the core rays vary, though in certain selections there is a fair degree of uniformity. In selection 1228s–25 (fig. 4, A) the rays are uniformly narrow and very numerous. Sometimes the rays are so close together that the bundles coalesce and one sees only numerous xylem rows embedded in small-celled parenchyma. The number of rays is not bound up with the size of the core (fig. 5, A, B, and fig. 6, A), although large cores often give a greater peripheral count because of the splitting off of secondary rays, which, however, do not reach the center. Occasionally the number of core rays is very small, as is shown in Figure 4, B. The absence of a readily seen relationship between the number of rays and the weight and percentage of sucrose is shown in Table 6. As in Table 5 the beets are arranged in series; those of the first group (selection No. 898–24) are arranged from low to high percentage of sucrose, those of the second group (selection No. 1569–24) from low to high weight, and those of the third group (selection No. 7794–24) from small to large neck diameter. Originally nine groupings were made, but since the different selections behaved alike, only the data of three groups are shown. (Table 6.)

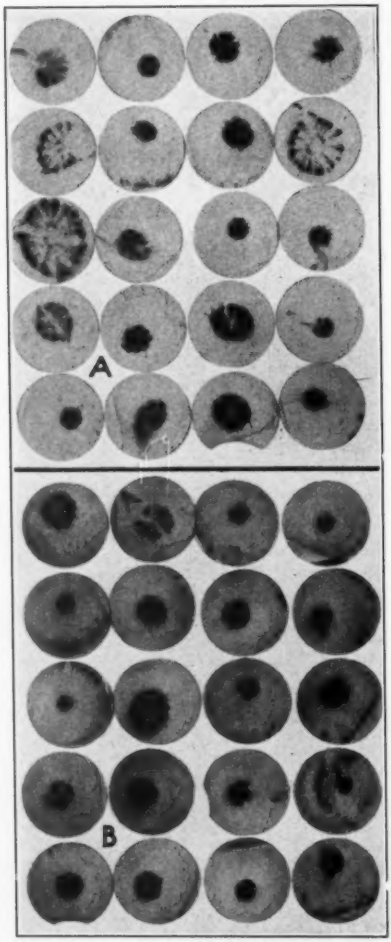


FIGURE 3.—Cross sections from central cores of sugar beets, arranged according to weight from left to right with first section from lightest beet. × 1½ A, Selection No. 2445s-25; B, selection No. 554-24

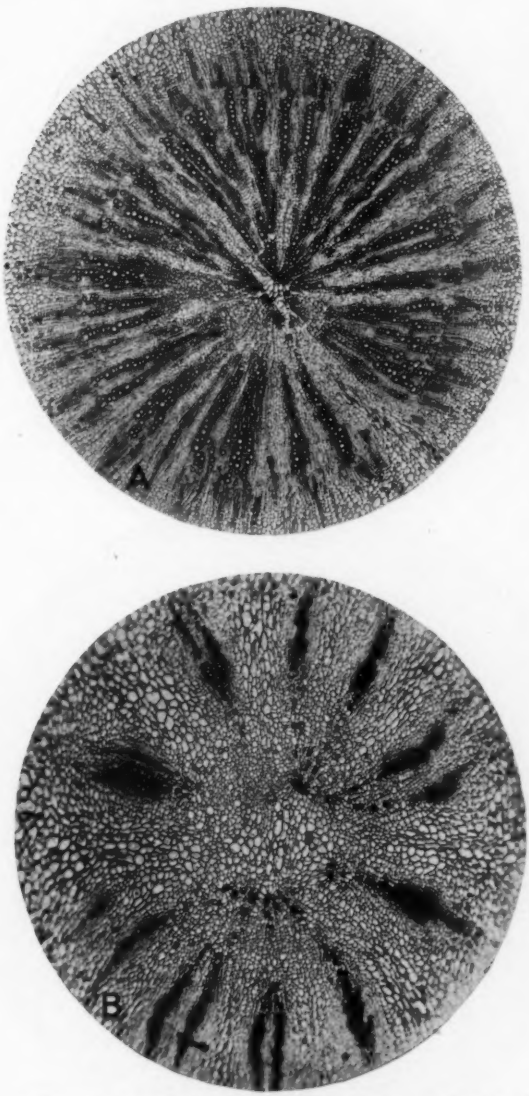


FIGURE 4.—Cross sections of cores of sugar-beet selections. A, No. 1228s–25. × 16. B, No. 1845s–25. × 10. Note the radical difference between the structure of the two cores

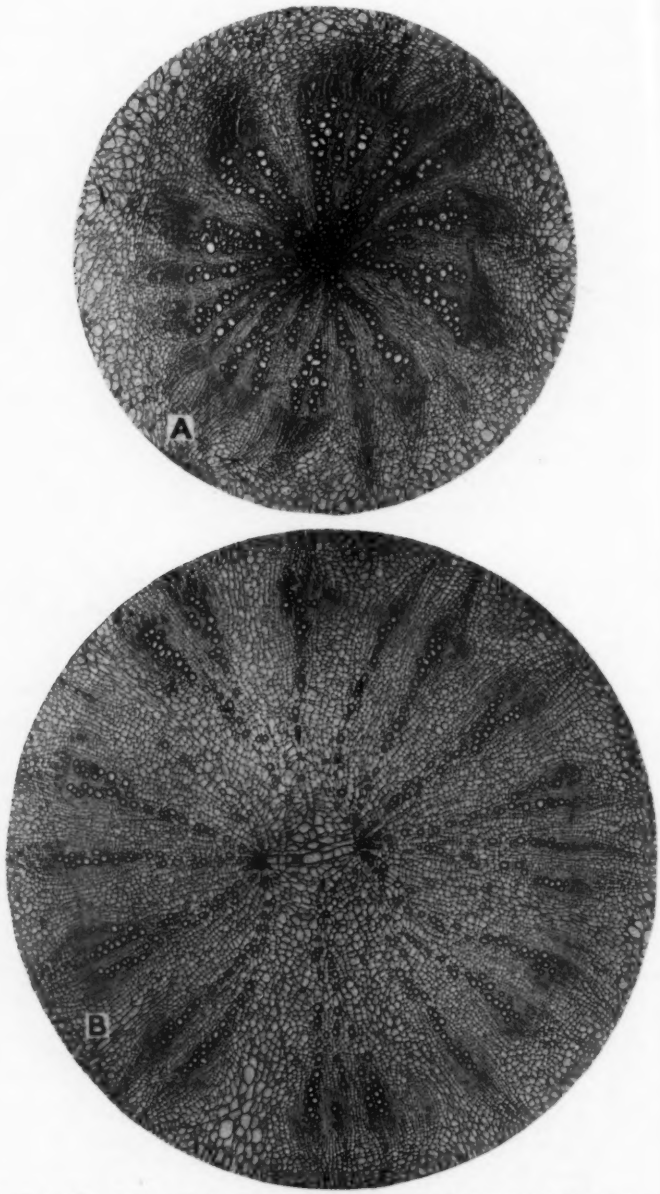


FIGURE 5.—Cross sections of two sugar beets of selection No. 1845s-25 with 15.8 per cent sugar content. × 19. A, Small dense core; B, larger, more or less open core. The bundles are very symmetrical and have well-developed sheaths

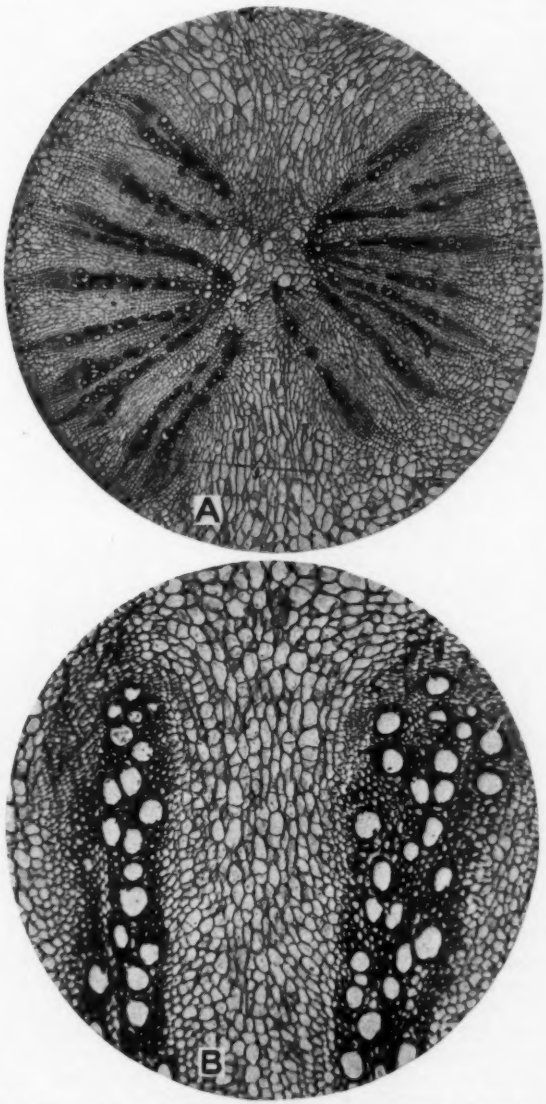


FIGURE 6.—Cross sections of sugar-beet selections. A, Central core of No. 1845s-25 with 11.2 per cent sugar content. Note the narrow, very symmetrical rays. × 16. B, Bundle of central core of No. 4621-22 with most of the sugar sheath lignified. × 63

TABLE 6.—*Vascular strands of core rays in central core of three beet selections grown at Fort Collins, Colo., 1928*

Item	Neck end		Tap end	
	Diameter of core	Rays	Diameter of core	Rays
Selection No. 898–24 (arranged in series from low to high sugar content)	*Mm.*	*Number*	*Mm.*	*Number*
	8	13	5	17
	7	12	5	16
	3.5	13	5	13
	5	16	2	14
	5	8	4	13
	5	15	3	13
	5	13	5	16
	3	13	4	14
	4	13	3.5	16
	4	14	4	15
	3.5	13	6	21
	4	17	3	17
	3.5	14	4	16
	4	11	5	17
	3	8	4	14
	5	15	5	22
	4	13	4	15
	5	18	4	16
	5	15	5.5	20
	6	16	4	19
	5	11	4	15
	6	12	6	16
	4	10	4	12
	3.5	12	2	18
	4	11	4	17
Mean	4.6	13	4.2	16
Standard deviation	1.4	2.30	1.03	2.47
Coefficient of variability	30.43	17.69	24.52	15.44
Selection No. 1569–24 (arranged in series from low to high weight)				
	4	18	3	22
	5.5	19	4	20
	3.5	13	2	18
	2.5	15	3	18
	5.5	14	3	15
	5	13	2.5	19
	3	12	2.5	16
	3	12	3	14
	6	11	3.5	17
	3	14	2.5	19
	3	13	2.5	19
	6	14	3	16
	3	15	3	16
	4	12	2	22
Selection No. 1569–24.	*Mm.*	*Number*	*Mm.*	*Number*
	6	15	2	19
	3.5	13	3.5	18
	4	11	4	18
	6.5	16	3	17
	6	13	3	21
	5	15	3	18
	4	15	3	18
	4	14	2	15
	7	18	3	23
	4.5	11	2.5	19
	4	11	4	15
Mean	4.4	13.9	2.9	18
Standard deviation	1.29	2.18	.58	2.54
Coefficient of variability	29.32	15.68	20.00	14.11
Selection No. 7794–24 (arranged in series from small to large neck diameter)				
	2.5	10	3.5	14
	3	19	4	19
	2.5	18	3.5	19
	3	10	3	13
	4	10	3.5	14
	3.5	16	4	22
	3.5	12	4.5	20
	6	16	4	18
	4.5	15	5	20
	3	9	3	15
	2.5	16	3	18
	2	15	2	17
	3	17	5	22
	2	10	3	20
	4	15	3.5	19
	4	13	3.5	16
	5	22	5	25
	4	17	4	17
	4	11	4	16
	2.5	12	3	16
	2	15	2	22
	6	17	4	19
	2.5	14	3	15
	3.5	11	3.5	16
Mean	3.3	13.8	3.4	17
Standard deviation	1.16	3.21	.82	2.93
Coefficient of variability	35.15	23.26	24.11	17.23

STRUCTURE OF THE VASCULAR RING

The vascular zone of each ring is composed of numerous collateral bundles separated from one another by medullary-ray tissue of varying width. Secondary rays frequently develop inside the bundles, causing them to split and making it difficult, in bundle counts, to obtain a correct estimate of the number of bundles per unit of distance. In longitudinal section the bundles of a ring anastomose tangentially, forming a network of unequal meshes. (Fig. 7, A–C.) Furthermore, Dostál (*3*), in a recent study on tangential polarity in beets, has shown that the course of these anastomosing bundles is somewhat dextrorse for the outer rings, sinistrorse for the inner rings, and straight radial for the intermediate rings. This asymmetrical ana-

tomical development, according to Dostál, is reflected externally by the somewhat spiral course of the lateral beet depressions containing the root traces.

The relative width of the vascular tissue was determined from freshly cut sections, because of the color contrast which the latter afford and which disappears in preserved material. An examination of a number of individual selections indicates that vascular prominence or conspicuousness is a property of a selection and is independent of beet size. There will, of course, be a variation in the

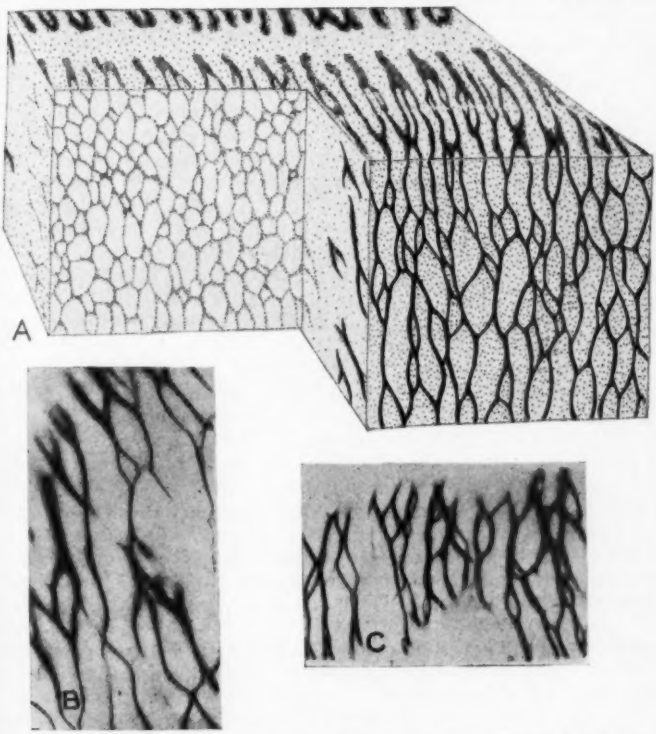


FIGURE 7.—Anastomosing of vascular region of sugar beet. × 2 A, Drawing of fourth ring; B, photograph of tangential section through xylem; C, similar section from a different part of the beet

width of the vascular rings of large and small beets, but the relative proportion between width of vascular tissue and width of interzonal parenchyma will remain constant. Vascular-ring prominence and percentage of sucrose do not seem to be related. A broad vascular ring associated with narrow interzonal parenchyma is not necessarily indicative of high sugar content, for sometimes individual beets with inconspicuous rings have a higher percentage of sugar than beets with prominent rings, a fact well illustrated in Plates 1, B, and 2, B. It must be admitted, however, that in this case the beet illustrated in Plate 1, B, had a higher ring-density factor than the second beet.

MICROSCOPIC CHARACTERS

Comparative anatomical studies on the structure of the sugar beet date back practically to the beginning of beet culture, though the most extensive phytotomic studies were made at the close of the nineteenth century at the various sugar-beet breeding stations.

Schindler and Proskowetz (*10*) in their pioneer studies on beet races also made an investigation of the anatomy of three races—Klein Wanzleben, Vilmorin Rose Hâtive, and Vilmorin Blanche Améliorée. They determined for 10 uniform individuals of each race the number of rings, the number of vascular bundles, and the size of the interzonal parenchyma cells. The counts were made in the peripheral region of the neck, and the size of the area studied had a radial and tangential extent of 1 cm. and 0.5 cm., respectively. Although counts were made at three different places, the areas involved were too small to yield reliable data, so that the observational error could easily have been big enough to account for the differences in the data obtained. Schindler and Proskowetz concluded that the beets rich in sugar possess a larger number of lignified bundles, and therefore more lignin, and a smaller celled interzonal parenchyma than beets poor in sugar.

Schneider (*11*), questioning the results of Schindler and Proskowetz, maintained that the number of xylem-containing bundles is no index to the amount of lignin, and that Schindler's method of counting the bundles is misleading, since bundles that had only a single xylem cell were counted, while those without xylem cells were omitted. Schneider reinvestigated the results of Schindler and Proskowetz, but he used in his studies an even smaller number of beets than they had used. He examined the varieties Vilmorin Blanche and Chotetovka. Like Schindler, he made his counts in the peripheral region of the neck but extended the radial depth of the areas to 1.77 cm. Schneider found that the variety rich in sugar had fewer rings, fewer vascular bundles, fewer but wider and thicker vessels, and fewer lignified parenchyma cells than the variety poor in sugar. According to Schneider, beets rich in sugar are less woody than those poor in sugar, a view that is in harmony with observations of De Vries (*14*) and opposed to those of Schindler.

TABLE 7.—*Total and lignified bundles in rings of Klein Wanzleben and Imperial varieties (according to Kraus)*

Item	Number of bundles per unit area in ring No.—									
	1	2	3	4	5	6	7	8	9	10
Klein Wanzleben:										
Total	8.7	9.6	11.0	14.6	14.6	17.4	17.9	14.3	21.0	23.7
Lignified	7.7	8.4	7.3	8.7	6.1	6.4	6.3	4.1	.7	.2
Imperial:										
Total	8.5	13.0	15.5	18.8	19.7	19.0	20.3	19.6	22.7	26.0
Lignified	7.1	8.0	8.4	9.4	9.0	8.8	5.6	4.7	5.2	.....

Kraus (*7*) counted the bundles in the different rings of the varieties Imperial and Klein Wanzleben. The counts were made on six individuals of each variety and the width of the areas measured was 2 mm. Table 7 gives the averages of his results for total and lignified bundles. These two varieties show differences in the number of total and of

lignified bundles. In Klein Wanzleben the number of bundles increases centrifugally, while the number of lignified bundles decreases in the same ratio. In the Imperial variety this condition is less pronounced. Kraus is inclined to think that the smaller the number of bundles the greater is the degree of lignification within a bundle.

Since a correlation between the percentage of sucrose and the anatomical structure was the primary object of all phytotomic studies, the localization of the sugar in the different tissues was investigated and special correlative studies of tissues rich in sugar were made. More than 50 years ago Wiesner (*15*) and De Vries (*14*) showed that the small-celled parenchymatous tissue surrounding the bundles was especially rich in sugar. This tissue was subsequently named "sugar sheath" by De Vries. The sugar-sheath cells, in addition to being smaller than the surrounding parenchyma cells, have thicker walls. The walls of the sugar-sheath cells are of cellulose and ordinarily do not change even in mature beets, but occasionally they become lignified. Lignified sugar-sheath cells are found individually or in groups, and in extreme cases the entire sheath may be lignified. (Fig. 6.) At a later period Peklo (*9*), studying the distribution of the sugar by the phenylhydrazine method, discovered that the largest osazone precipitate was found in the sieve tubes, and that therefore the phloem was to be considered the tissue especially rich in sugar. Geschwind (*4*) examined the development of the sugar sheath in 500 beets and came to the conclusion that while it is possible to separate rich beets from poor beets on the basis of differences in the development of the sugar sheath, subsequent breeding experiments as reported by Hoffmann (*5*) showed these results to be of no practical significance. The same can be claimed to some extent for the work of Peklo, since he was not always able to correlate massiveness of phloem with sugar content of the individual.

THE FOURTH VASCULAR RING

In examining the bundles of the various vascular rings it is apparent that the oldest, i. e., the innermost ring, has the smallest number of bundles per linear dimension and that the peripheral ring has the largest. In other words, the number of bundles increases centrifugally. Furthermore, it will be noted that in any given ring the spacing of the bundles is not equal and the size of the bundles also shows a certain degree of variation. This is due to the fact that the bundles anastomose profusely tangentially, so that a given bundle will have a different appearance in different sections. To obviate this difficulty it is necessary to count the bundles over a sufficiently large area, and since, because of the great labor involved, it is impracticable to count the bundles in all the rings, it was considered best to make a more complete study of the fourth ring and carry it through for the individuals of all the selections studied. But even then it is impossible to arrive at some simple representative figure, since it is left to the observer to evaluate what is an individual bundle. In some beets the bundles are fairly uniform and distinct, but in others it is often difficult to decide whether a certain bundle is a component of a large bundle or a unit in itself. (Fig. 8.) Even if one succeeds in eliminating as far as possible the personal equation, direct comparisons of bundle counts may be of little value, since some

individuals possess characteristically large bundles, while in others they are small, although quite distinct.

From the data presented in Table 2 it will be seen that each beet has its own number of bundles; however, if the individuals of different groups are compared there appears to be no doubt that in certain selections, for example, No. 3956–24, the number of bundles is consistently low; in others it is much higher. Generally speaking, a large beet has fewer bundles per unit area than has a small beet, but this relation does not always hold true. There appears to be no distinct relation between number of bundles and percentage of sucrose. This

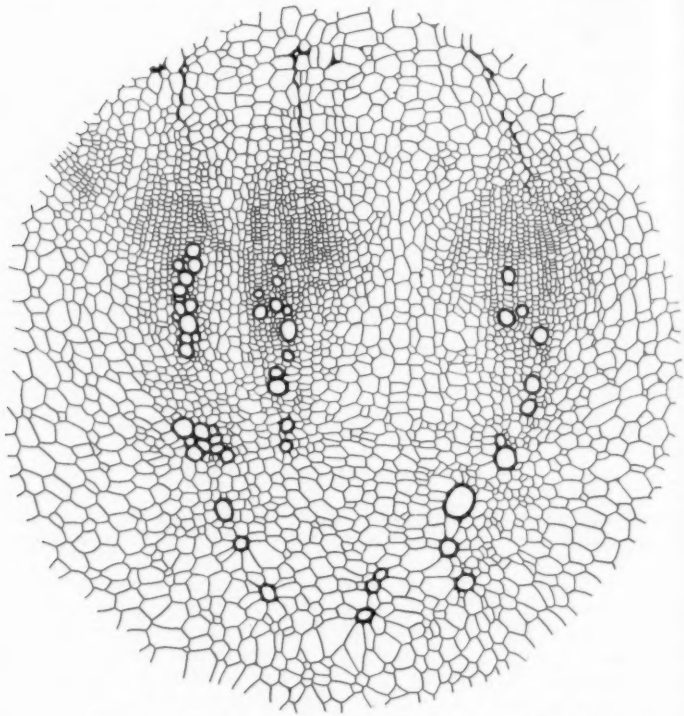


FIGURE 8.—Large bundle from fourth ring of selection No. 2287–24. × 70

is true in individuals of the selection and in individuals of different selections.

The structure of the individual bundles, except for size and degree of maturity, is alike. (Fig. 9.) The bundles are widest in the region of the cambium and taper gradually toward the phloem and xylem poles. This gives them the appearance of a double wedge. (Fig. 10, A.) The xylem is formed of one or several disconnected rows of vessels, surrounded by a sheath of elongated small-celled parenchymatous tissue—the sugar sheath of De Vries. The phloem is composed of sieve tubes, companion cells, and phloem parenchyma, is

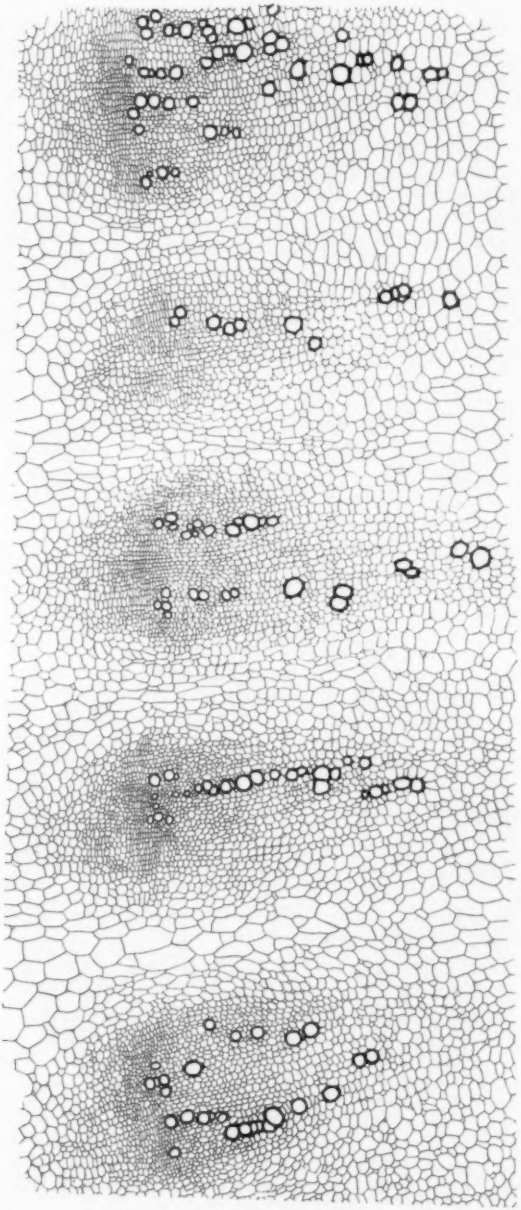


FIGURE 9.—Partial cross section of fourth ring. × 85

FIGURE 10.—Cross sections of sugar-beet vascular bundles. A, Rich beet of selection No. 4621-22. ×45. B, Small, very rich beet from Rocky Ford, Colo. Note large amount of phloem. ×100

commonly well developed, and exceeds in mass the xylem. This is especially true of the bundles of the more peripheral rings.

The existence of variation in size of the bundles has already been mentioned. The difference in size may extend to either the radial or the tangential dimension. One frequently observes in beets with a low percentage of sucrose a rather elongated xylem pole surrounded by a very narrow zone of sugar-sheath cells. In beets high in sucrose, on the other hand, the sugar sheath is much broader, although the amount of xylem may be less than in the first type. Certain selections, especially No. 3403–24 (Table 2), are characterized by a very narrow vascular ring. (Fig. 11, B.) This same selection also illustrates the fact that narrow-zoned individuals have not necessarily a small number of vessels; in fact, the number in some individuals may be quite large. (Table 8.) The xylem cells of the individual bundles vary normally in size. The cells of greatest diameter are commonly found near the xylem pole and those of smallest diameter near the cambium. In individuals that are low in sucrose, however, there is no reduction in the size of the xylem cells of the cambium region, a fact recorded by many of the earlier investigators. This, however, is not to be considered a hard and fast rule, since in bundles of poor beets small xylem cells may be found in the cambium region; whereas, conversely, bundles in rich beets may have large xylem cells near the cambium. Aside from normal variations of xylem cells within a bundle there is also a general variation in size when different individuals are compared. This is well illustrated in Figure 12. In A, B, and D of that figure the xylem cells appear of the same size, but in C the small diameter of the cells is notable.

TABLE 8.—*Number of xylem cells per centimeter as counted in bundles of fourth ring of sugar-beet selections grown at Fort Collins, Colo., 1927 and 1928*

1927									1928		
No. 8753–24	No. 6008–25	No. 3403–24	No. 1591–24	No. 193R–25	No. 2310s–25	No. 4621–22	No. 2445s–25	No. 2287–24	No. 8753–24	No. 6008–25	No. 3403–24
182	296	248	-------	365	198	158	215	263	229	220	235
285	463	211	143	244	274	187	330	183	267	205	148
276	199	-------	135	198	250	231	157	205	165	210	190
306	292	-------	207	218	159	174	220	278	267	149	215
213	166	197	104	243	217	150	170	237	251	167	212
237	260	125	185	334	274	218	300	208	223	240	140
---	280	-------	149	189	259	383	246	230	186	220	169
255	254	-------	149	160	-------	-------	200	268	200	195	276
324	251	202	176	233	198	191	276	267	228	230	278
230	250	150	170	197	240	215	231	100	170	210	249
247	360	235	143	295	210	-------	154	226	-------	-------	-------
233	202	231	154	309	198	185	182	-------	-------	-------	-------
---	226	238	99	233	345	-------	178	243	-------	-------	-------
171	332	237	220	378	205	110	293	145	-------	-------	-------
232	190	-------	170	200	131	160	301	140	-------	-------	-------
212	385	178	-------	260	159	216	163	173	-------	-------	-------
138	387	158	-------	242	227	255	198	138	-------	-------	-------
300	237	258	108	201	161	298	140	216	-------	-------	-------
187	300	170	122	300	212	238	210	194	-------	-------	-------
267	235	222	219	236	148	175	233	155	-------	-------	-------
247	249	120	174	-------	232	-------	225	156	-------	-------	-------
180	200	363	180	-------	177	-------	234	282	-------	-------	-------
218	346	-------	216	-------	356	-------	177	150	-------	-------	-------
---	270	136	177	-------	111	-------	244	198	-------	-------	-------
338	258	186	107	-------	260	-------	182	283	-------	-------	-------
Mean..	239	275	203	159	251	216	208	218	204	219	205	211

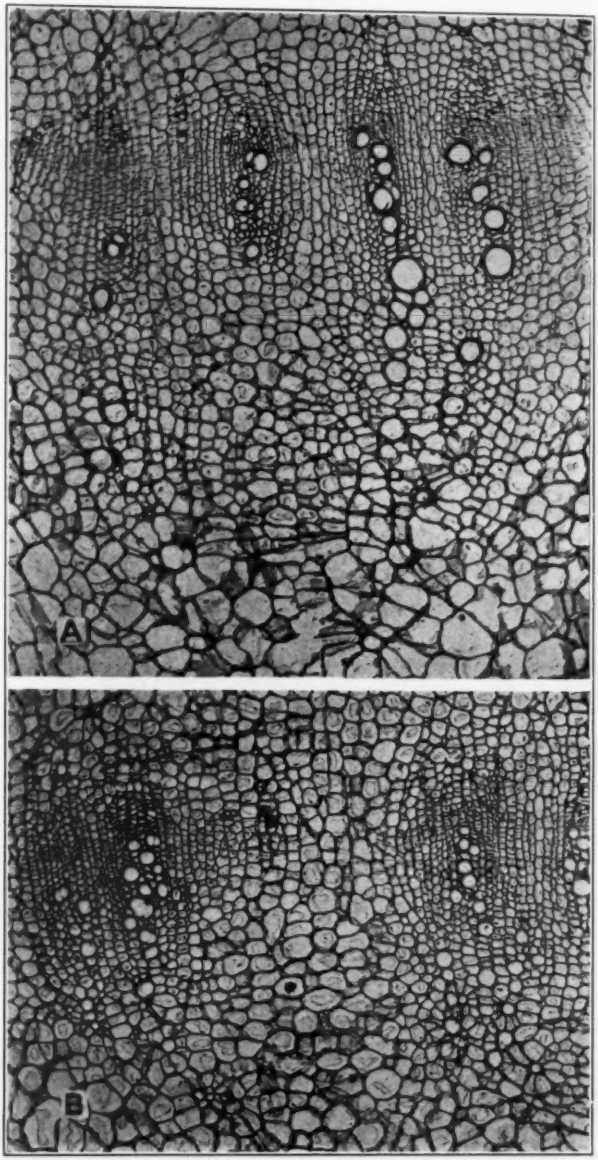


FIGURE 11.—Cross sections of vascular bundles of sugar-beet selections. × 50. A, No. 5942-24 with 11.3 per cent sugar content; B, No. 3403-24 with 14.1 per cent sugar content. Note extreme narrowness of vascular bundles

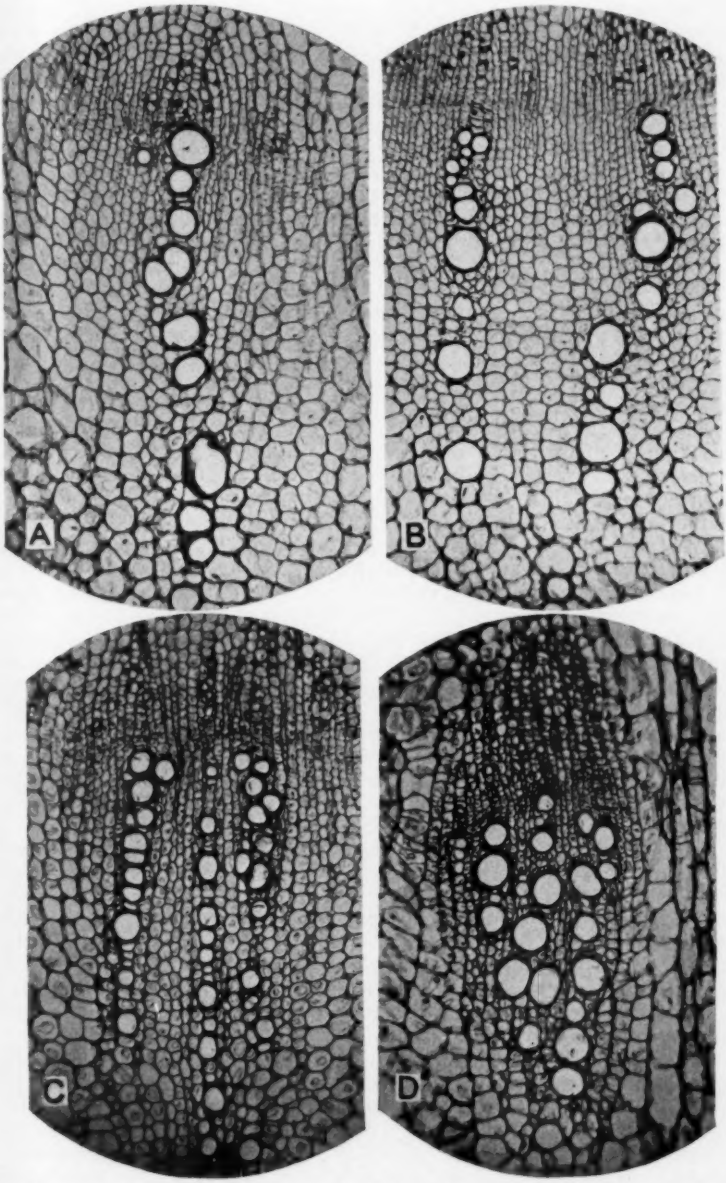


FIGURE 12.—Cross sections of vascular bundles of sugar-beet selections. × 95. A and B, No. 3942-24. Sugar content of A, 11.3 per cent; of B, 13.6 per cent. C, No. 1228s-25; sugar content, 12.1 per cent. D, No. 1161-24; sugar content, 11.8 per cent

In many selections the xylem consists only of vessels of varying sizes, but in some there are also a larger or smaller number of lignified sheath cells. (Figs. 6, B, and 13, B.) These cells appear singly, in small groups, or in large areas. Not all individuals of a selection possess them, and while their presence is not necessarily correlated with sugar content, there is a tendency for rich beets to have a smaller number of these lignified cells than poor beets, or they may be absent altogether in the better types.

Just as the count of vascular bundles is subject to error, an evaluation of the amount of lignified tissue is even more so. In bundles having only a few vessels the size of the individual cells does not differ greatly, and counts in individuals of the same type give at least comparative data. However, in the case of many vessels a count of the actual number is misleading, since many of the "supernumerary" vessels are usually small in diameter and do not contain the amount of lignin found in large vessels. The phloem part of the vascular bundles is usually well developed, which is especially noticeable in the peripheral rings where xylem elements are often still undifferentiated. It would appear from the investigations of Peklo (*9*) that the phloem is the seat of highest sugar concentration, and its relative amount should therefore be directly correlated with the sugar content; and, indeed, very rich beets, as illustrated in Figure 10, A, have a very massive phloem. The investigations of Peklo have never been repeated, and they are only roughly approximated in connection with the present anatomical studies.

Beets with rather wide vascular zones were cut in slices about one-fourth inch thick and the slices divided along the cambium line of the mature rings. The samples were analyzed separately but showed practically no difference in regard to sugar distribution. It would appear that either the phloem tissue is not so high in sugar as was claimed by Peklo, or, as is more likely, the accumulation of sugar in the phloem is counterbalanced by the sugar stored in the sheath cells surrounding the xylem. It has been shown previously that a wide vascular ring does not always denote a rich beet, but if individuals with a massive phloem should polarize low, one would usually not have to look far to find an explanation.

The vascular bundles of the central core reflect in a large measure the appearance of the bundles of the mature rings. (Fig. 14.) The core bundles are in general more lignified than the mature ring bundles, a condition that is very striking in small compact cores. The vessels of the core have a tendency to be large, especially in individuals with a low percentage of sucrose. In rich beets they are not only of smaller size but suffer a further reduction in the region of the cambium. Geschwind (*4*), who examined the central core of the taproot end of the beet, noticed the same tendency.

The interzonal parenchyma proper comprises a relatively narrow zone of parenchymatous tissue. Actually, however, the zone seen in looking at cross sections of a beet comprises three regions: A centrifugal, more or less broad band containing scattered xylem cells; an intermediate, purely parenchymatous zone; and an inner region containing obliterated phloem. Since earlier investigations associated a small-celled parenchyma with a high percentage of sucrose, an attempt was made to verify such a correlation in the selections under

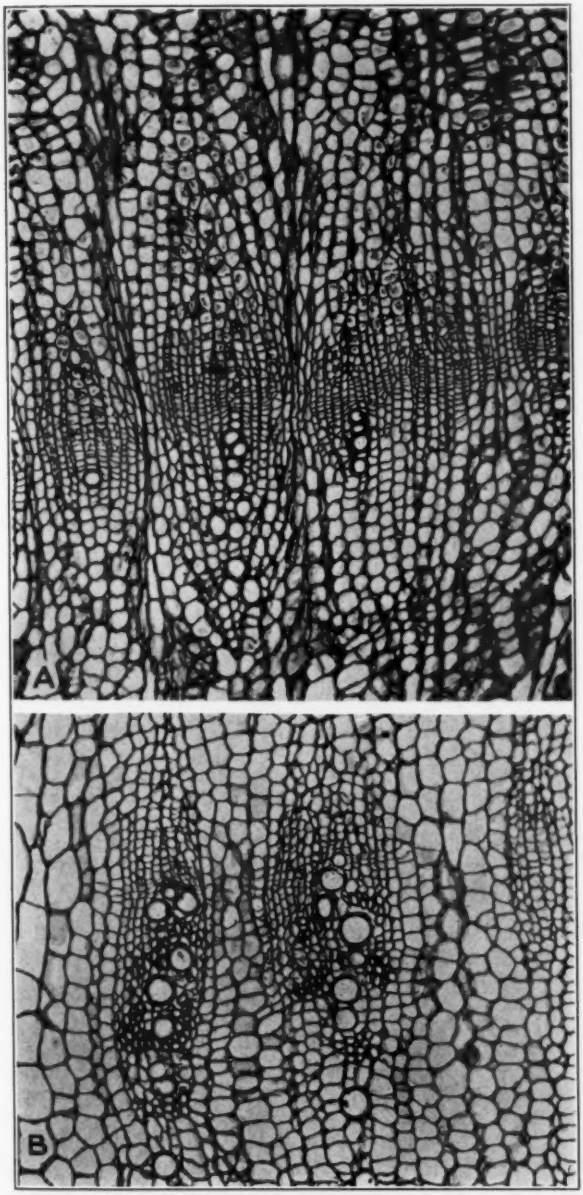


FIGURE 13.—Cross sections of vascular bundles of selections of sugar beets from Rocky Ford, Colo. × 110. A, Very rich beet with phloem large in comparison with xylem. B, No. 1973, with 12.5 per cent sugar content. Note lignified sugar-sheath cells and large size of xylem in comparison with A

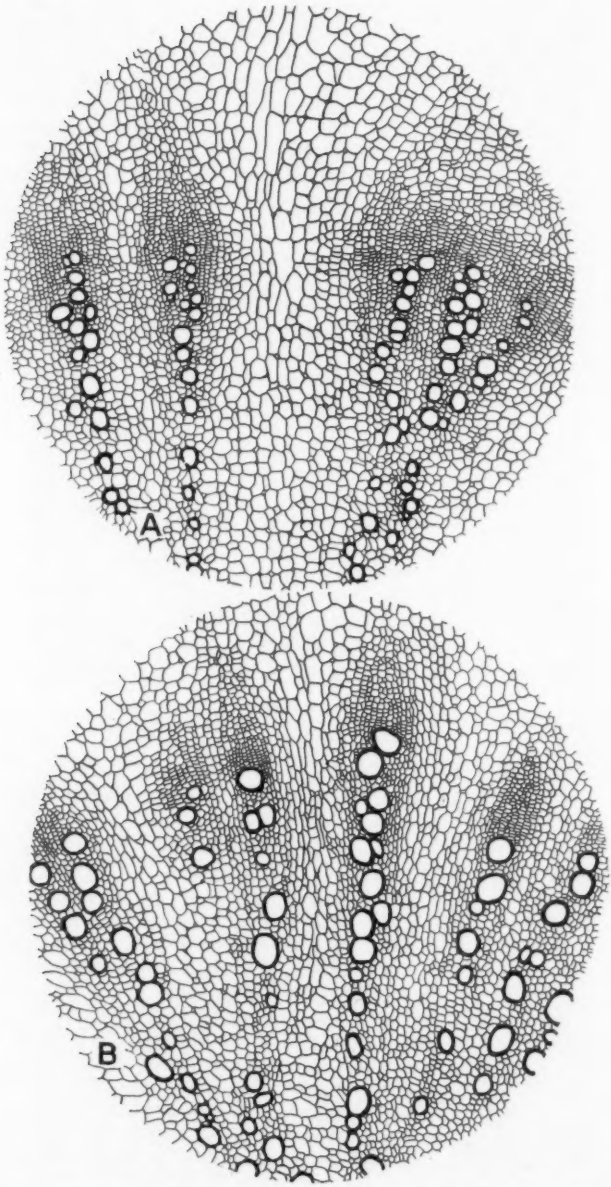


FIGURE 14.—Cross sections of vascular bundles of central cores of sugar beets. ×60. A, Shown in Figure 5; B, shown in Figure 6, A

observation. It was found difficult to approximate correctly the size of these cells. Individual measurements were considered inadequate, and in counting the cells of a given region one has to contend with errors arising from the false impression made by cells that have been cut through the corners and thus appear small. Everything considered, however, the method of projecting a slide on a white background and counting the cells was found to be most satisfactory. Table 9 gives data on individuals of three selections. The data are arranged in a series from low to high sugar.

TABLE 9.—*Number of interzonal parenchyma cells per square millimeter in fourth ring of sugar-beet selections grown at Fort Collins, Colo., 1927*

No. 4545-24		No. 2445s-25		No. 2287-24	
127	176	95	163	92	83
99	120	96	130	65	56
102	139	101	151	62	60
126	131	176	97	82	83
133	125	144	150	96	115
129	97	97	128	72	87
121	119	156	91	78	133
108	145	99	125	81	137
132	128	183	133	117	76
123	102	183	129	55	94
94	146			71	84
				61	67
Mean...123		Mean...131		Mean....83	

While there is a great deal of variation and no apparent connection between the size of parenchyma cells, the weight of the beet, and the percentage of sucrose, the large-celled parenchyma in No. 2287–24 is related to the large central core and broad first ring of this selection, and it is not unlikely that some of the other selections might show similar relationships.

EFFECT OF ENVIRONMENT ON ANATOMICAL STRUCTURE

The summer of 1928 differed from that of 1927 in being uniformly sunny, with rains falling in the right amount and at the right time for maximum sugar production. The increase in sugar, which averaged 2 per cent more than in 1927, was reflected to a certain degree in a changed anatomical structure.

A study of Table 2 shows that, although the weight of the beets fluctuates indifferently in the two seasons, characters such as ring number and width of central core and first ring appear to reflect very definitely seasonal differences. The average ring count is, on the whole, conspicuously and consistently higher in 1928 than in 1927, except for a small number of selections where the ring number did not change. In selection No. 4545–24 (Table 3) the average ring count was 11 for both seasons; selection No. 5656–24 showed a similar conformity, but there was a greater variation in the weight of the beets; selection No. 3956–24 stands out preeminently in both seasons because of its large ring number; selection No. 1161–24 gave a low ring count in both years, but the coefficient of variability was slightly larger in the first year. The ring-density coefficient showed a fair degree of uniformity when individual selections were compared.

Selection No. 1228s–25 had the lowest ring-density coefficient, that is, less than 2 in both years. In selection No. 4545–24 the coefficient reached the high figure of 2.7 and 2.6 for the two seasons. In selection No. 3956–24 the coefficient was also very high, which was quite in harmony with the large ring number.

The size of the first ring and central core showed great seasonal variations, but there was noted a certain degree of uniformity in the individual selections, which seasonal effects were unable to obscure. Selections Nos. 554–24, 3956–24, and 1293s–25 (Table 3) had a small first ring in both seasons. In some of the large-ringed types the diameter of the first ring remained above a mean of 20 mm. in both years, although there was a general tendency toward a smaller ring in the second year. The same tendencies were observed in the central core. The small-core types, selections Nos. 554–24, 730–24, 8146–24, and 6077–24 (Table 3) showed the same small core in both seasons, while most of the large-core types suffered an unequal decrease in size the second year.

The number of vascular bundles per centimeter, with few exceptions, was smaller in 1928 than in 1927. The same tendency was observed in the xylem vessels, although actual counts were not made for all selections. These data are in harmony with the finding that the percentage of sucrose was higher in 1928 than in 1927, although it must be remembered that within the different selections there exists little if any correlation between number of bundles and percentage of sucrose. The fact that, regardless of the smaller number of bundles and xylem vessels, the 1928 beets appeared somewhat more woody than the 1927 crop may be accounted for by the relatively greater abundance of lignified sugar-sheath cells. Although these cells were not found in many selections, those that showed them in 1927 developed them even more abundantly in 1928. In certain selections, notably Nos. 7794–24, 4545–24, and 2445s–25, the lignification in the sugar sheath was very extensive.

RELATION OF STRUCTURE TO PERCENTAGE OF SUCROSE AND TYPE PURITY

In summing up the results of their extensive phytotomic studies on sugar beets, the early investigators conceded that while it was possible to separate rich and poor beets on account of single anatomical differences, it was hopeless to try to distinguish between beets that differed only slightly in their percentage of sucrose; and since even positively classified beets performed in a contradictory fashion the second season, it was concluded that anatomical studies had no practical significance in beet-breeding work. The investigations reported in this paper only emphasize the fact that it would be futile to use a single character as a criterion for percentage of sucrose, especially in dealing with a mixed population. To exemplify: Of four different selections of sugar beets that were recently received from Rocky Ford, Colo., the beets of two of the selections had the same broad vascular zones, and similar narrow first rings and small central cores, but while one sample tested 17 per cent the other tested only 12.5 per cent. Wherein did they differ, and which structural configuration put one type in the poor and the other in the rich class? The beet rich in sugar had 13 rings to the 10 of the low-sucrose beet. The

central core of the rich beet had fewer rays and the vascular rings fewer bundles than the poor beet. A microscopic study showed further that a large part of the sugar-sheath cells of the poor beet were lignified. On the other hand, there appeared to be little difference in the expanse of the phloem tissue and the development of interzonal parenchyma. The larger ring number and the slightly greater ring-density coefficient appeared to be the only features that separated the rich from the poor beet, for the presence of lignified sugar-sheath cells, though suggestive of poor quality, would not have been a safe criterion, since another beet which tested only one-half of 1 per cent higher showed no lignification.

The practical beet grower would probably have relied on the color differences of the flesh to classify the two types, and the numerous observations made in the course of these studies, which have been referred to earlier, give support to the belief that there exists a correlation between flesh color and sugar content. In the present case the flesh of the rich beet was a uniform white, while that of the poor beet was a watery yellow. The beets from the fourth sample were comparatively small and tested very high. They had, of course, a high ring-density coefficient, but the most distinctive character was the prodigious development of the phloem tissue, as can be seen in Figure 10, A and B.

The studies on the different beet selections have shown that the anatomical configuration associated with high or low sugar is not necessarily the same in all selections. Broad vascular zones are of little significance if associated with a small ring number or watery flesh; on the other hand, broad interzonal parenchyma may not necessarily be indicative of poor quality. Undoubtedly, a large ring number, a high ring-density coefficient, broad vascular zones associated with narrow bands of interzonal parenchyma, a fairly large number of evenly spaced bundles with well-developed phloem, a moderate number of xylem vessels, and absence of lignification in the sugar sheath are all indicative of high sugar. The relative development of any of these characters may be different in the different selections, and their influence on the percentage of sucrose in a given selection can be ascertained only through systematic study. Since uniform performance can be safely looked for only in relatively pure types, sugar-beet breeding experiments should take cognizance not only of the factors that express high sugar content but also of those that guarantee type purity. The breeding experiments of recent years have been fruitful in securing types of uniform leaf and root characters, and although at present many of these pure strains are not high producers, there are others that compare favorably with the best commercial stock. The anatomical study of a number of such selections has shown that structural differences exist which in some instances are sufficiently well marked to characterize a selection. In other types well-marked tendencies were observed which seasonal influences partly obscured but did not obliterate.

It is safe to predict that once the different types have become, through continuous inbreeding, sufficiently homozygous to assure uniformity in subsequent generations, it will be possible to give their anatomical type picture, which would more closely delimit them than could be done by external morphological characters alone.

A knowledge of the particular anatomical configuration of each type would not only aid in keeping the type pure, but would expedite the selection process through the elimination of such mother beets as showed distinct anatomical differences. If, for example, there is within a selection a strong tendency toward the development of a small core, the elimination of large-core mother beets should aid in securing more uniform seed, especially where group selection is already practiced.

Additional studies carried on over a number of years must demonstrate which characters in the different selections can be relied on to serve as a criterion for purity of type as well as to determine the actual relation to sugar content. Since climatic factors influence the performance of the crop, and since performance and structure are for physiological reasons interrelated, the effect of a different season must be reflected in a changed structure; yet these influences probably do not have more than a quantitative value and should not obliterate the trustworthy type characters. Other environmental factors which often affect the yield should have little influence in these selection studies, since through proper management their effects should be reduced to a minimum.

The different beet selections are more or less sharply defined morphologically by their leaf and root characters, while structurally they reflect certain peculiarities which are more strongly realized in some selections than in others. The possibility of a causal relationship between gross morphology and inner structure suggests itself only to be answered negatively after a study of Tables 1, 3, and 10.

TABLE 10.—*Yield of 30 sugar-beet selections grown at Fort Collins, Colo., 1927*

Selection No.	Sucrose	Sugar per acre	Beets per acre	Selection No.	Sucrose	Sugar per acre	Beets per acre
	Per cent	*Pounds*	*Tons*		*Per cent*	*Pounds*	*Tons*
150–24	15.53	4,584	14.75	1845s–25	14.84	4,764	16.05
193R–25	15.03	4,156	13.19	2287–24	15.14	4,350	14.36
554–24	15.24	3,953	12.97	2310s–25	14.53	4,936	16.98
600s–25	12.73	1,413	18.66	2346s–25	14.64	5,575	19.03
730–24	14.53	4,815	16.56	2447s–25	15.03	4,658	15.48
808–24	14.64	5,170	17.66	3403–24	13.53	4,396	16.23
992s–25	15.24	3,525	11.56	3956–24	13.94	4,714	16.89
1161–24	14.53	3,800	13.06	4545–24	15.03	3,843	12.78
1228s–25	14.13	4,299	15.20	4621–22	15.14	4,294	14.17
1293s–25	14.64	3,824	13.06	5656–24	15.05	4,612	15.95
1427–24	13.44	4,386	16.23	5942–24	15.34	4,838	15.77
1569–24	13.13	3,921	14.92	6077–24	14.24	3,857	13.53
1591–24	15.03	4,714	15.67	7794–24	12.84	3,834	14.92
1612–24	15.03	4,714	16.14	8146–24	13.74	4,801	17.47
1818s–25	14.53	5,072	17.28	8753–24	14.74	4,815	16.33

The various selections differ widely in their outer appearance and show no definite connection between root and foliage characters. Spreading or erect leaf habit may be associated with large or small leaf area, and this character in turn may have no relation to the number of leaves characteristic of the different selections. It must be admitted at the outset that a relationship between outer appearance and inner structure exists only for individuals of a given selection and that it is unsafe to extend the characterization to other selections. Slender elongated roots may have either a large or a small first ring or central core, or a small or large ring number. Poor selections, characterized

by a small ring number, appeared at first glance to be associated with a comparatively small leaf area; yet the best selections, as judged by the number of rings, also had a small leaf area. The shape of the root shows little consistent relation to the inner structure. In one selection a parsnip-shaped root was associated with a large central core, while in another selection of that type both core and first ring were small.

These results only emphasize the necessity of studying the anatomical configuration of all established and genetically important types in order to know the inherent tendencies, to preserve them, and to extend their usefulness along desired lines.

SUMMARY

An anatomical study of 30 sugar-beet selections, comprising 1,700 individuals, indicates that the structural configuration of the different selections, for certain characters at least, is specific, and suggests the possibility of obtaining for those strains which through continuous selection have become homozygous an anatomical type picture which seasonal effects may alter but not obliterate and which would aid in delimiting a type and keeping it pure. In order to obtain such type pictures it is necessary to examine entire cross sections of as many beets of a selection as can be obtained, although in case of prospective seed beets the measurements must necessarily be limited to a median section of a cork-borer sample through the central neck region.

Most of our cultivated sugar beets have ivory-white flesh but in poor strains it is of a watery greenish or yellowish hue. The sharpness of the zonation as seen in a cross section is bound up with the color of the flesh, being least marked in pure-white individuals and most prominent in poor, watery ones. The relative width of the vascular zones and the interzonal parenchyma varies indifferently, though in some of the selections the vascular tissue forms conspicuously broad rings.

The number of mature rings is about one-half of the total number produced. The latter is consistently and conspicuously high in some selections and relatively low in others, but in most of the selections examined marked differences were not found. A high total ring number is frequently correlated with a high mature number, but occasionally the opposite is true. The relative ring width of the mature rings is usually related to the weight and slightly to the ring number of the beet, but this correlation is absent in beets with a relatively small ring number.

The ring-density coefficient can be considered an index to percentage of sucrose, except where ring number or ring width, for some reason or other, shows no relationship. The size of the central core and first ring is in certain selections uniformly large or small, so that aberrant forms can be easily detected and eliminated. The size of the central core is related neither to the percentage of sucrose nor to the weight of the beet, nor does the number of core rays show any relation to these factors, except that in very large cores the number of rays is apt to be high.

Although vascular-ring prominence and sugar content are in general independent variables, in beets where broad vascular zones are associated with a high ring-density coefficient a high sugar content is

usually assured. The number of vascular bundles of a ring bears no relation to the percentage of sucrose and only a slight relation to beet size, since as a rule a large beet will have fewer bundles per unit distance than a small beet. The number of xylem cells shows an even greater variation and the data are less reliable, since the size of the cells varies greatly. There appears to be no connection between number of xylem cells and percentage of sucrose. A certain number of selections showed the presence of a smaller or larger number of lignified sugar-sheath cells. These selections were hard to section but were not necessarily poor in sugar, although within a selection there was a tendency to develop a larger amount of lignified sheath cells in poor beets than in rich ones. While the phloem tissue is well developed in most beets, very rich individuals always have a very massive phloem.

The size of the interzonal parenchyma cells shows no important correlation with percentage of sucrose, but may be related within a selection to the width of the rings.

In comparing beets grown in the seasons of 1927 and 1928, it was found that those grown in 1928 had in general a higher number of rings, a smaller central core, and a smaller first-ring diameter than those grown in 1927. Since the differences were only of degree, and since the different selections reacted similarly, type characters were not obliterated. In 1928 the beets averaged higher in percentage of sucrose than in 1927. This increase in sugar appears to be reflected structurally in a higher ring number and a relatively smaller central core and first ring.

The anatomical type picture appears to vary in different beet selections, and although the structural features associated with high sugar appear to be understood, the ideal structural configuration is not always the same. Although performance and structure are interrelated phenomena, the study of any one character may not give a correct evaluation of the sugar-storing capacity of a beet, since the same character in a new combination may influence performance in a different way. This may explain why, for example, beets with comparatively narrow vascular zones are not necessarily poor sugar producers.

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TOXICITY OF BIKUKULLA FORMOSA (WESTERN BLEEDINGHEART) [1]

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INTRODUCTION

Three different native plants of the genus Bikukulla (also known as Dicentra) have previously been studied in this laboratory and reported on with respect to their poisonous properties and danger to grazing animals.[2] One of these, *B. cucullaria*, was found to contain an intensely poisonous alkaloid and to be a grave danger to animals on ranges where it may occur. The other two, *B. canadensis* and *B. eximia*, although found to contain alkaloids in appreciable quantity, could be classed as harmless, since their basic contents were not toxic. Still another member of this group of plants, *B. formosa*, has been found growing wild in the West and is the subject of the present paper.

BOTANICAL DESCRIPTION OF BIKUKULLA FORMOSA

Bikukulla formosa (Andr.) Coville. Western bleedingheart. (Fig. 1.) A smooth, succulent perennial with thick fleshy branching rootstock; stemless; flowering stalks several, 6 inches to 2 feet high; flower clusters twice branched, slightly taller than the leaves; leaves twice ternately dissected, long-stemmed; corolla flattened, ovate-cordate at the base, the four petals 7 to 9 lines long, united up to the middle, withering persistent about the seed pod, crests of inner petals tubular, conspicuous; sepals two, scalelike; stamens in two sets of three each; stigma one, 2-lobed; seed pod 1-celled.

Shady woods of British Columbia (where first discovered by Menzies), south through the lower mountain woods of western Washington and Oregon, the coast ranges of California to Alameda County, and in the Sierra Nevada to Tulare County. More at home in the moist climate from British Columbia to Oregon.

CHEMICAL EXAMINATION

Although most of these plants have been the subject of more or less chemical study, little is known concerning the physiological properties of their constituents. Apparently all of them contain alkaloids, but in few instances have these been investigated physiologically.

The alkaloids of *Bikukulla formosa* were studied by Heyl,[3] who, working with roots of plants obtained from one of the German botanical gardens, was able to isolate protopine and two other crystalline alkaloids. Protopine is a well-known alkaloid found among the numerous constituents of opium and also in a variety of plants.

[1] Received for publication Dec. 31, 1929; issued May, 1930.

[2] BLACK, O. F., EGGLESTON, W. W., KELLY, J. W., and TURNER, H. C. POISONOUS PROPERTIES OF BIKUKULLA CUCULLARIA (DUTCHMAN'S-BREECHES) AND B. CANADENSIS (SQUIRREL-CORN). Jour. Agr. Research 23: 69–78, illus. 1923.

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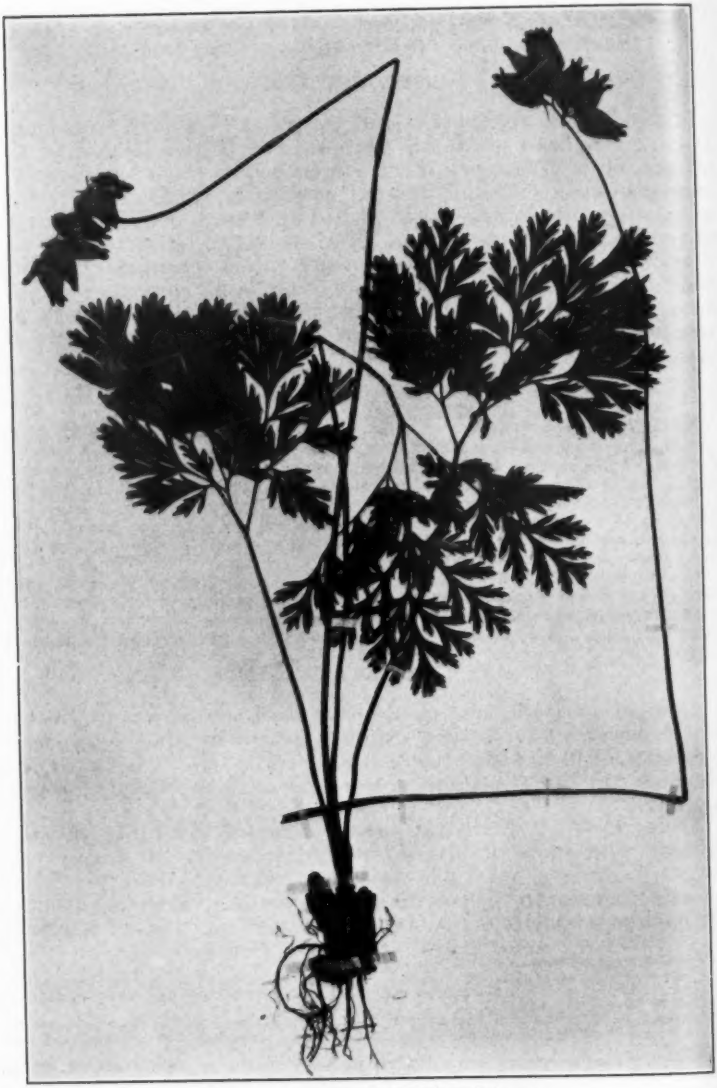

FIGURE 1.—Flowering plant of *Bikukulla formosa*

It is physiologically active, producing local anesthesia and, in mammals, narcosis and convulsions, but it has no extensive use in medicine. The other two alkaloids were not named by Heyl, and nothing is known concerning their physiological properties.

In June, 1928, a quantity of *Bikukulla formosa* was collected in the Cascade Range about Fish Lake ranger station, Santiam National Forest, Oreg. The tops were dried and sent to Washington, D. C., where they arrived in fair condition. Eight hundred grams of this material, ground to a moderate fineness, was packed in a percolator and moistened with 80 per cent alcohol weakly acidified with acetic acid. After standing 24 hours it was exhaustively percolated with alcohol of the same strength, and the solution thus obtained was concentrated by vacuum distillation to about 500 c. c. A moderate quantity of 10 per cent lead acetate solution was added, the precipitate filtered, and the excess of lead removed by dilute H_2SO_4. The resulting solution was partially neutralized with solid $CaCO_3$, then concentrated to a volume of about 300 c. c. This extract was made strongly alkaline with Na_2CO_3, resulting in a voluminous precipitate, which was removed by filtration and the filtrate shaken out twice with ether. Further treatment with ether and other solvents failed to remove any further basic material, showing that the extraction had been complete.

The precipitated bases and residues from the ether extractions, which dried to a brown sirupy material, were united and treated with hot benzol, which left a slight quantity of black tarry substance undissolved. The crude basic material prepared by this procedure weighed 8.3 gm., or just over 1 per cent of the plant material. Heyl [4] stated that he obtained 3 per cent of crude alkaloid, but as he worked with the roots alone, the difference between his results and the writers' may well have been due to the presence of a much greater percentage of alkaloid in the roots than in the tops.

The crude basic material, when freed from the solvent and dried, consisted of a light-brown amorphous gummy mass which grew darker in color on standing in the desiccator and responded to all the ordinary tests for alkaloids. It showed no tendency to crystallize, although Heyl reports that under the same conditions his material yielded crystals of protopine. The crude preparation described was subjected to a number of methods of purification, but neither through these nor by the use of any of the ordinary solvents or combinations of such solvents could any well-defined separation or crystallization be brought about.

PHYSIOLOGICAL TEST OF THE EXTRACT

Since the main object of this investigation was to gain a practical knowledge of the toxicity of the plant to animals, the crude basic material afforded a starting point. Two-tenths of a gram was dissolved in very dilute hydrochloric acid and the volume made up to 5 c. c. A white mouse weighing 21 gm. was given 0.25 c. c. of this solution by subcutaneous injection. The animal remained in a quiescent, semistupified condition for 15 minutes and was then seized with convulsions which lasted intermittently for 30 minutes,

[4] HEYL, G. Op. cit.

when it died. The lungs were collapsed, the extremities blue, the heart distended, and death was probably due to respiratory paralysis. By gradually diluting the solution, progressively weaker doses of the base were administered to mice in the same way until a dilution was reached that failed to prove fatal. In all cases the symptoms of poisoning were the same.

From this procedure it was determined that the lethal dose of the crude alkaloids lies between 5 and 2.5 mgm. for a mouse of about 20 gm. in weight; or, calculated on the basis of percentage of alkaloids in the plant, it would be represented by from one-half to one-fourth gram of dry plant.

Although the toxicity of the plant to mice does not prove that it is equally poisonous to other animals, it is evident that *Bikukulla formosa* should be placed in the category of poisonous plants and should be regarded as a potential danger to livestock wherever it is found.

FERRIC CITRATE AS AN INGREDIENT OF MINERAL MIXTURES IN PAIRED-FEEDING EXPERIMENTS WITH GROWING SWINE[1]

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INTRODUCTION

During the summer of 1927 there was undertaken at the Illinois Agricultural Experiment Station a critical study of the ingredients of mineral mixtures in the ration of growing swine. The methods adopted for use in this study differ fundamentally from those employed in previous experimental work on the question. The feed intake of each pig was under control throughout the experiment, as all pigs were fed in individual feeding crates. The pigs were fed in pairs, so that for each pig receiving the supplement under investigation there was a strictly comparable pig which resembled it closely at the start of the experiment and consumed the same amount of feed throughout. At the present time the work is being confined to a study of definite chemical compounds superimposed singly upon an otherwise well-balanced, practical ration, not particularly rich in the mineral elements contained in the supplement used.

Because of its frequent use in mineral mixtures, ferrous sulphate (copperas) was the first compound to receive attention. Iron is known to be required in animal nutrition, although most farm feeds contain this element in extremely small concentration. The results of this first test, as well as a discussion of the advantages of the paired method of feeding, have already been published.[3]

METHODS

On account of the negative nature of the results of feeding additional iron in the form of copperas it was decided to test another compound of iron, the thought being that possibly a ferrous salt would not be as suitable as a ferric salt. Ferric citrate was the compound selected to be fed in the second test. The methods of procedure were those outlined and used in the preceding test. Briefly, they consisted of feeding both pigs of each pair the same amount of a well-balanced ration, containing no feed conspicuously high in iron. One pig received only the basal ration, while the other received the same amount of the basal ration and in addition 3 gm. daily of ferric citrate in aqueous solution.

The pigs were paired on a basis of weight, sex, blood lines, thrift, vigor, type, and prospective outcome. Ten pairs of pigs were fed. They were all pure-bred Duroc-Jerseys raised on the University farm. Their weights varied from 64 to 90 pounds at the beginning of the

[1] Received for publication Dec. 3, 1929; issued May, 1930.
[2] Grateful acknowledgment is made to F. J. McClure for the results on the iron and red-cell content of the blood.
[3] CARROLL, W. E., and MITCHELL, H. H. RESULTS OF FEEDING COPPERAS IN PAIRED-FEEDING EXPERIMENTS WITH GROWING SWINE. Amer. Soc. Anim. Prod. Proc. 1927: 73-82. 1928. (See also STUDY INGREDIENTS IN COMPLEX MINERAL MIXTURES. Ill. Agr. Expt. Sta. Ann. Rpt. 41: 132-136. 1928.)

test, though no two pigs of a pair varied initially more than 5 pounds in weight and most of them weighed within 2 or 3 pounds of each other.

The basal ration used was the low-iron ration of the previous test and consisted of yellow corn, soybean-oil meal, linseed meal, alfalfa meal, calcium carbonate, and sodium chloride. The proportion of the ingredients was changed as the pigs increased in weight. The changes made are indicated in Table 1.

TABLE 1.—*Variations in the proportion of the ingredients of the ration fed the growing swine at different live weights*

Feeds	Proportion in which the feeds were mixed for pigs weighing—		
	100 pounds or less	100 to 150 pounds	Over 150 pounds
Ground corn	70	80	90
Soybean-oil meal	18	12	6
Linseed meal	6	4	2
Alfalfa meal	6	4	2
Calcium carbonate	1	1	1
Sodium chloride	0.5	0.5	0.5

The chemical composition of the feeds used throughout the experiment, determined upon composite samples, is given in Table 2.

TABLE 2.—*Percentage chemical composition of the feeds on the fresh basis*

Feeds	Dry substance	Crude protein	Ether extract	Ash	Crude fiber	Nitrogen-free extract	Iron
Soybean-oil meal	88.56	42.54	5.60	6.04	5.84	28.54	0.0291
Corn	86.64	8.27	3.39	1.38	2.43	71.17	.0085
Linseed meal	89.95	34.44	5.05	5.39	8.91	36.16	.0153
Alfalfa meal	88.98	15.31	2.03	7.13	27.29	37.22	.0249

The pigs were fed twice daily in individual feeding crates. The mixed ration was weighed to one-tenth of a pound and fed in one end of a divided metal trough with which each crate was equipped. Water was available in the other end of the trough. The feeds were given dry except that the solution of ferric citrate was poured over the ration of the test pigs just before the pigs were turned into their crates. The pigs were watched closely in an attempt to keep the feed consumption up to the limit of the appetite of the lightest feeding pig of each pair.

Except while feeding, the pigs were kept on concrete floors, the check pigs being kept in one group and the pigs receiving iron in another. Each group was given a pen in the northwest side of a central swine barn adjacent to which was an outside concrete runway.

The pigs were continued on feed until one or both pigs of a pair reached a final weight of 175 pounds.

RESULTS

The results of this test are summarized briefly in Table 3, which gives for each pig the initial and final weights;[4] the weekly, the total,

[4] Initial and final weights are averages of weights on three consecutive days.

and the average daily gains; as well as the total feed eaten, the average daily ration, and the feed eaten per pound of gain.

TABLE 3.—*Weights, gains, and feed consumption of 10 pairs of pigs, one of each pair being on the basal ration alone and the other getting a ferric citrate supplement*

	Pair 1		Pair 2		Pair 3		Pair 4		Pair 5	
	Check pig	Pig on ferric citrate	Check pig	Pig on ferric citrate	Check pig	Pig on ferric citrate	Check pig	Pig on ferric citrate	Check pig	Pig on ferric citrate
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Final weight	175	170	175	159	181	182	174	164	181	175
Initial weight	86	82	80	75	70	72	67	64	90	88
Total gain	89	88	95	84	111	110	107	100	91	87
Period on test (days)	84	84	98	98	106	106	98	98	85	85
Average daily gain	1.06	1.05	0.97	0.86	1.05	1.04	1.09	1.02	1.07	1.02
Gain or loss in weight:										
Week 1	0	−2	0	−3	3	−2	1	−2	0	−2
Week 2	5	6	5	6	6	4	6	7	6	7
Week 3	5	6	6	3	3	5	5	5	4	3
Week 4	6	8	9	8	8	8	8	6	7	6
Week 5	9	7	5	10	8	10	9	8	9	10
Week 6	6	6	9	7	6	9	9	10	9	9
Week 7	10	11	10	7	8	6	8	9	9	6
Week 8	8	6	7	8	7	7	9	8	7	9
Week 9	10	13	12	11	11	10	9	7	12	10
Week 10	11	6	10	9	3	2	3	5	9	8
Week 11	8	9	7	9	10	10	11	9	6	12
Week 12	11	12	9	5	9	9	7	11	* 13	* 9
Week 13			−1	0	8	10	9	8		
Week 14			7	4	8	9	13	9		
Week 15					* 13	* 13				
Total feed eaten	315	315	351	351	412	412	367	367	323	323
Average daily ration	3.74	3.74	3.58	3.58	3.88	3.88	3.74	3.74	3.80	3.80
Feed per pound of gain	3.53	3.58	3.69	4.18	3.71	3.74	3.43	3.67	3.55	3.71

	Pair 6		Pair 7		Pair 8		Pair 9		Pair 10	
	Check pig	Pig on ferric citrate	Check pig	Pig on ferric citrate	Check pig	Pig on ferric citrate	Check pig	Pig on ferric citrate	Check pig	Pig on ferric citrate
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Final weight	172	174	174	169	188	178	176	180	176	167
Initial weight	83	87	78	82	78	74	72	71	69	66
Total gain	89	87	96	87	110	104	104	109	107	101
Period on test (days)	84	84	91	91	113	113	120	120	126	126
Average daily gain	1.06	1.04	1.05	0.96	0.97	0.92	0.87	0.91	0.85	0.80
Gain or loss in weight:										
Week 1	1	0	−2	1	−1	−2	0	−1	1	−1
Week 2	6	6	3	1	7	8	6	5	5	8
Week 3	8	4	5	3	2	1	1	1	5	2
Week 4	4	6	7	9	9	7	6	5	7	7
Week 5	7	9	10	7	8	6	7	9	8	6
Week 6	9	6	6	6	6	6	9	8	−1	−1
Week 7	8	9	10	9	6	8	9	8	5	6
Week 8	8	7	6	4	9	6	6	7	6	5
Week 9	12	14	12	10	10	11	10	10	7	7
Week 10	7	7	12	8	3	3	8	9	5	8
Week 11	9	8	7	11	11	10	−2	4	7	8
Week 12	10	11	11	15	6	10	2	3	6	3
Week 13			9	3	11	9	6	5	3	4
Week 14					4	5	6	6	5	6
Week 15					9	10	8	7	9	13
Week 16					* 10	* 6	11	10	11	9
Week 17							* 11	* 13	10	8
Week 18									8	3
Total feed eaten	317	317	349	349	421	421	417	417	398	398
Average daily ration	3.78	3.78	3.84	3.84	3.73	3.73	3.48	3.48	3.16	3.16
Feed per pound of gain	3.56	3.65	3.64	4.01	3.83	4.05	4.01	3.83	3.72	3.94

* 8 days in this period. Initial and final weights are the averages of 3 weights taken on consecutive days. Single weights were used in the other cases.

It is a matter of no surprise that the rates of gain are less than the ration is capable of supporting under conditions of unrestricted feeding. Obviously the prime requisite in a feeding experiment such as this is to obtain closely comparable controls. Submaximal feeding and gaining are necessary consequences of proper experimental control and do not detract from the significance of the comparisons made, since a ration deficient in some particular constituent for growth should be improved in its growth-promoting properties at all levels of feeding by the addition of the proper supplement.

As would be expected, there was considerable variation in both rate and economy of gain among the various pairs. The difference between pigs of the same pair, however, was not great. The largest difference in both rate and economy of gain existed between the pigs of pair 2, a difference of 12.1 per cent in rate and 12.5 per cent in economy of gain.

The check pig in every case, except pair 9, made more rapid and economical total gains than the test pig that ate the same amount of feed and in addition received 3 gm. of ferric citrate daily throughout the test. The results suggest, as did the copperas results of the preceding test with reference to a tankage ration, that added iron salts may depress the rate of gain of growing pigs. However, a further analysis of the data by statistical methods indicates that only an inconsiderable probability has been established for this interpretation.

The 10 pairs of pigs were on experiment for from 12 to 18 weeks. By considering each week for each pair an experimental datum, there are 143 pair weeks, affording as many comparisons of test and control pigs. If the iron supplement were without value in promoting growth and without harmful effect in retarding growth, the chances are even that in any one week on equal intakes of food the gain in weight of the pig receiving iron will exceed that of its control mate, or will be less than that of its pair mate. With 143 comparisons, therefore, the ideal result, if the iron citrate supplement were without effect, would be 71.5 favoring the check pig and 71.5 favoring the test pig. If the weekly gains in Table 3 are consulted, it will be found that 79.5 comparisons favor the check pig, and 63.5 favor the iron-fed pig. The ideal result of 71.5 would of course be a very unlikely outcome in a negative experiment. The question at issue is whether the deviation from this ideal obtained in this particular experiment, i. e., $79.5-71.5=8$, could reasonably result from the operation of chance, which would have determined it if the iron supplement were without effect on growth. This is a proposition in which the assessment of probabilities may be made by means of the binomial distribution.[5] The standard deviation of the frequency distribution of the outcome of 143 events, each of which may result with equal probability in either of two ways, is given by the expression $\sqrt{0.5\times0.5\times143}$, which is equal to 5.98. The deviation of 8 from the ideal outcome is only 1.34 times this standard deviation. It would occur by chance about once in six trials, and hence its occurrence in any one trial is not indicative of the operation of any but chance factors in the determination of the outcome of the weekly comparisons of the gains of pair mates.

[5] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, rev. and enl., p. 63. Edinburgh and London. 1928.

THE COMPOSITION OF THE BLOOD

The iron content and the concentration of erythrocytes were determined upon the blood of 7 of the 10 pairs of pigs, the blood samples being taken from the tail at the conclusion of the feeding experiment. Iron was determined by the Kennedy method.[6] The results will be found in Table 4.

TABLE 4.—*Iron and erythrocyte contents of the blood of the pigs in 7 of the 10 pairs at the conclusion of the feeding test*

Pig	Pair 2	Pair 3	Pair 4	Pair 7	Pair 8	Pair 9	Pair 10
ERYTHROCYTES IN MILLIONS PER CUBIC MILLIMETER							
Control	6.40	9.14	6.01	7.47	6.43	6.87	8.12
Ferric citrate	7.64	9.25	8.29	8.04	7.49	7.23	8.58
PER CENT OF IRON							
Control	0.0455	0.0504	0.0436	0.0406	0.0450	0.0400	0.0414
Ferric citrate	.0478	.0556	.0480	.0424	.0455	.0484	.0479

For each of the seven pairs, the pig receiving ferric citrate was found to have blood containing a greater concentration of red blood cells and a greater concentration of iron than its control mate. A consistent outcome of seven such comparisons as these would, according to the binomial distribution

$$\left(\frac{1}{2}+\frac{1}{2}\right)^7$$

occur by chance only twice in 128 trials, and a consistent positive effect, considering the probability of a depressing effect of iron to be nil, only once in 128 trials. Hence, the observations are highly indicative of an effect of supplemental iron on the hemoglobin and red cell content of the blood.

On an average, the blood of the pigs given ferric citrate contained 8,070,000 red cells per cubic millimeter as compared with 7,210,000 for the control pigs, a difference of 11.9 per cent. The average iron content of the blood was 0.0479 per cent for the pigs given ferric citrate and 0.0438 per cent for the control pigs, a difference of 9.4 per cent. The pigs receiving no iron supplement were not anemic,[7] so that the slightly higher cell count and iron (hemoglobin) content of the blood in all probability induced by the daily administration of 3 gm. of ferric citrate, although of scientific interest, should be accorded no considerable practical importance. In this connection it may be recalled that Whipple has maintained dogs in an anemic condition at one-third the normal hemoglobin level for four years in perfect health and activity.[8]

[6] KENNEDY, R. P. THE QUANTITATIVE DETERMINATION OF IRON IN TISSUES. Jour. Biol. Chem. 74: 385–391, illus. 1927.

[7] For example, the red-cell count of the pig is given as 6,200,000 per cubic millimeter in the following publication: MARLOFF, R. DIE FRÜHEREN ZÄHLUNGEN DER ERYTHROCYTEN IM BLUTE VERSCHIEDENER TIERE SIND TEILWEISE MIT GROSSEN FEHLERN BEHAFTET. Pflüger's Arch. Physiol. 175: 355–370. 1919. The red-cell count is given in the following as 6,215,000 red cells per cubic millimeter as the average obtained from 25 pigs weighing about 100 pounds: PALMER, C. C. MORPHOLOGY OF NORMAL PIGS' BLOOD. Jour. Agr. Research 9: 131–140. 1917.

[8] WHIPPLE, G. H. EXPERIMENTAL ANEMIAS, DIET FACTORS AND RELATED PATHOLOGIC CHANGES OF HUMAN ANEMIAS. Jour. Amer. Med. Assoc. 91: 863–867, illus. 1928.

SUMMARY

The administration of ferric citrate at the rate of 3 gm. daily to young growing pigs on a ration of yellow corn, soybean oil meal, linseed meal, alfalfa meal, calcium carbonate, and sodium chloride had no effect upon the rate or economy of gains in 12 to 18 weeks of feeding. The paired-feeding method was used in this trial, involving 10 pairs of pigs.

In seven pairs of pigs, the blood was examined at the conclusion of the feeding experiment. Among these pairs, the pig receiving iron consistently showed a higher red-cell count and a higher iron content of the blood. These results are statistically significant. Their practical importance, however, is inconsiderable, since the pigs not receiving iron were not anemic. There is no reason to believe that the iron supplement conferred any actual benefits upon the pigs receiving it.

THE PRODUCTION AND CURE OF NUTRITIONAL ANEMIA IN SUCKLING PIGS [1]

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INTRODUCTION

Although the investigation described in the preceding paper (*2*),[2] as well as contemporary work in this country (*1*), has not shown that good rations for young growing pigs are commonly deficient in iron even though they contain no feeds conspicuously high in this element, or that iron supplements to such rations will perform any useful purpose, the case of the suckling pig, subsisting largely or entirely upon the milk of the dam, may very well be in a different category. Although no record in the recent literature has been found of an iron analysis of the milk of the pig, the milk of cattle and of goats (*5*) contains only 0.0002 to 0.0003 per cent of iron, and human milk seems to contain but slightly more (*3*). As compared with other feeds (*17*), these milks, and presumably the milk of the pig also, are extremely poor in iron. Indeed, it has been found possible to induce severe nutritional anemia in rats (*19*), rabbits (*8*), and chickens [3] by the feeding of cow's milk. The probability of the occurrence of anemia in suckling pigs is thus not remote.

WORK OF OTHER INVESTIGATORS

The actual occurrence of anemia under farm and experimental conditions, and the symptoms by which it is accompanied, have been described by McGowan and his associates (*10*, *11*) in England, and by Doyle, Mathews, and Whiting (*4*) in this country. Its etiology, however, has been in doubt. McGowan claims that it is due to the feeding of iron-deficient rations to the sow, but Doyle and his associates were not able to confirm this belief. The latter investigators found that some factor in outdoor conditions tended to prevent anemia in suckling pigs. In a later communication (*13*) it was shown that sunlight was effective in preventing anemia, but that ultra-violet irradiation indoors was ineffective.

The remarkable finding of Hart and his associates (*9*), observed independently and less directly by McHargue and his coworkers (*12*), that the administration of copper salts to rats subsisting upon a milk diet prevented the appearance of the anemia that otherwise invariably occurred, afforded the first definite indication for the prevention and cure of nutritional anemia in suckling pigs. Hart himself was the first, in so far as the authors are aware, to apply this experimental finding to young pigs. In a talk given before the thirty-second annual meeting of the United States Livestock Sanitary Association in Chicago in December, 1928, Hart described his work on iron in nutrition,

[1] Received for publication Dec. 3, 1929; issued May, 1930.
[2] Reference is made by number (italic) to "Literature cited," p. 938.
[3] University of Illinois unpublished data.

and presented briefly the results of copper and iron medication of part of one litter of anemic pigs. The treatment was distinctly successful, but for some reason these results have not been included in the report of the paper appearing in the printed proceedings of the society.[4]

EXPERIMENTAL DATA

In a continuation of the work on the value of iron supplements in swine nutrition advantage was taken of this discovery of the function of copper in blood formation. Some preliminary observations on pigs farrowed from sows subsisting upon iron-poor rations (*14*) had not confirmed the belief of McGowan that anemia was a necessary sequel of such treatment. However, in the following year (spring of 1929) four Duroc-Jersey pigs from a litter of 13, raised under the usual routine at the swine barns, exhibited symptoms similar to those described by McGowan. Accordingly, samples of blood were taken from the tails of these pigs and analyzed for hemoglobin by the acid hematin method of Newcomer (*16*), the Newcomer plate being standardized by the Van Slyke oxygen capacity method (*18*).[5] The pigs were 11 days old, and the hemoglobin contents, expressed in grams per 100 c. c., was 5.05, 5.05, 5.57, and 5.05. Two of the pigs were then given daily by pipette a solution of copper sulphate containing 5 mgm. of copper daily and at the end of a week solutions of both ferric citrate and copper sulphate in amounts equivalent to 25 mgm. of iron and 5 mgm. of copper. One of these pigs died in about a week, but the hemoglobin in its blood had risen from 5.05 to 7.14 gm. per 100 c. c. The hemoglobin of the other treated pig remained at a low level for three weeks and then rapidly increased in the following three weeks to 10.1 gm. per 100 c. c., after which a slight drop occurred. The two untreated pigs showed a decrease in hemoglobin. At 25 days of age, one of them gave a sample of blood containing only 2.85 gm. of hemoglobin per 100 c. c. The iron and copper medication was started at this time, and the animal responded rapidly, so that at an age of 53 days the blood contained 9.62 gm. of hemoglobin per 100 c. c. The second untreated pig was kept in a distinctly anemic condition, with the hemoglobin varying from 2.5 to 3 gm. per 100 c. c., from the twenty-fifth to the sixtieth day of age. It was then given the usual daily dose of ferric citrate and copper sulphate. From a level of 2.96 gm. the hemoglobin increased in three weeks to 8 gm per 100 c. c. These pigs were all kept indoors.

This preliminary experiment confirmed in every way the experiment reported orally by Hart.

Observations were then made upon three litters of pigs that were cared for in the normal manner, no metallic salts being given. The only difference in the treatment accorded the litters was the time from birth at which they were removed outdoors into cindered pens. The first litter, 9 PC, was a vigorous one of four Poland China pigs farrowed on February 25, to which one Duroc-Jersey pig was later added. This litter was removed outdoors to a cindered pen on March

[4] A popular bulletin describing these and other results of a similar nature on suckling pigs has since appeared (*7*).

[5] Five standardizations with pig blood of the Newcomer colored glass disk gave an average of 0.003085 as the disk factor instead of 0.0035 as furnished by the manufacturers of the disk. All acid hematin solutions were prepared in the manner described by Newcomer and all were allowed to stand overnight at room temperature before readings were made. Turbidities, such as those encountered when acid hematin solutions are prepared from avian blood by the Newcomer procedure, were not encountered with pig blood.

15. Very soon after this one of the pigs, 9–30–B, escaped from the pen. Litter 4 PC was also a normal healthy litter of Poland China pigs, which on account of the prevailing bad weather was not removed to the outside until April 11, when the pigs were over 3 weeks of age. Litter 3 DJ was a small litter of Duroc-Jersey pigs which, when 2 weeks of age, appeared to be anemic. Blood samples were therefore taken at this time and for three succeeding weeks. On March 30 this litter was removed to an outside pen. The observations on the blood, including hemoglobin content, and red-cell counts on the first litter, are summarized in Tables 1, 2, and 3. In this and all subsequent experiments, blood samples were taken from an ear vein.

TABLE 1.—*Hemoglobin content and erythrocyte count of the blood of a vigorous litter of pigs*

[Litter 9 PC; [a] farrowed Feb. 25 and moved outside Mar. 15, no medication]

HEMOGLOBIN (GRAMS PER 100 C. C. OF BLOOD)

Date of observation	Age of pigs	Pig No.				
		9-9-S	9-90-S	9-30-B	9-3-S	2-9-S
1929						
Mar. 6	1 week 2 days	6.37	8.03			6.78
Mar. 13	2 weeks 2 days	6.64	7.86	7.80		7.01
Mar. 20	3 weeks 2 days	8.39	10.68	11.99		9.53
Mar. 27	4 weeks 2 days	9.87	11.11		10.90	12.21
Apr. 3	5 weeks 2 days	11.03	9.74		10.74	10.33
Apr. 10	6 weeks 2 days	11.47	10.56		12.91	12.84
Apr. 17	7 weeks 2 days	11.45	11.90		12.45	10.37

ERYTHROCYTES (MILLIONS PER CUBIC MILLIMETER OF BLOOD)

Mar. 6	1 week 2 days	5.06	5.52			6.26
Mar. 13	2 weeks 2 days	5.54	5.15	5.16		5.39
Mar. 20	3 weeks 2 days	5.42	6.63	7.65		7.34
Mar. 27	4 weeks 2 days	7.09	7.78		11.70	10.12
Apr. 3	5 weeks 2 days	8.53	7.85		8.53	7.09
Apr. 10	6 weeks 2 days	8.35	9.23		8.53	9.65
Apr. 17	7 weeks 2 days	8.47	7.57		9.44	

[a] Blood of sow 9 PC contained 9.65 gm. hemoglobin per 100 c. c. when tested Apr. 3.

TABLE 2.—*Hemoglobin in grams per 100 c. c. of the blood of a normal litter of pigs*

[Litter 4 PC; kept inside till Apr. 11; no medication]

Date	Age of pigs	Pig No.			
		4-30-B	4-30-S	4-3-S	4-90-S
1929					
Mar. 20	2 hours	9.31	9.05	11.25	
Mar. 27	1 week	4.00	4.05	(a)	5.40
Apr. 3	2 weeks	3.20	3.69		3.63
Apr. 10	3 weeks	2.55	6.82		3.83
Apr. 17	4 weeks	(b)	9.53		6.82

[a] Killed by sow. [b] Died.

TABLE 3.—*Hemoglobin in grams per 100 c. c. of the blood of a small, apparently anemic, litter of pigs*

[Litter 3 DJ; * farrowed Mar. 13; apparently anemic when study started on Mar. 27; sow and litter placed in outside pens Mar. 30; no medication]

Date	Age of pigs	Pig No.		
		3-30-S	3-30-B	3-99-B
1929				
Mar. 27	2 weeks 1 day	3.34	3.42	
Apr. 3	3 weeks 1 day	2.33	2.26	2.47
Apr. 10	4 weeks 1 day	9.36	7.40	8.95
Apr. 17	5 weeks 1 day	12.95	8.16	10.64

* Blood of sow 56 DJ contained 10.30 gm. hemoglobin per 100 c. c. when tested Apr. 3.

The main significance of these observations relates to the anemic condition developing in all pigs, no matter how healthy and vigorous they appeared to be, during indoor confinement, and the rapid alleviation of this condition upon removal to an outdoor cindered pen. According to the results obtained with litter 4 PC (Table 2), from which blood samples were taken two hours after birth, the pig is born with a fairly high level of hemoglobin, which is rapidly reduced during the first week of life.

The most striking illustration of the rapid decrease in the hemoglobin of pigs during the first few days after birth and of the rapid recovery that is effected by the change from indoor to outdoor conditions was obtained with litters 90 DJ and 93 DJ, farrowed from sisters bred to the same boar. These results are summarized in Tables 4 and 5. The hemoglobin measurements are presented graphically in Figure 1 A and B.

TABLE 4.—*Blood hemoglobin and erythrocyte count, and weights, of a litter of pigs kept outdoors*

[Litter 90 DJ;* sow and pigs moved outdoors in a cindered pen on the sixth day after farrowing; no medication]

HEMOGLOBIN (GRAMS PER 100 C. C. OF BLOOD)

Date	Age of pigs	Pig No.					
		90-3-B	90-3-S	90-30-S	90-9-S	90-99-S	Average
1929							
Apr. 5	6 to 10 hours	13.75	11.94	9.68	11.25	11.00	11.52
8	3 days	9.05	6.74	8.43	8.16	8.09	8.08
10	5 days	7.56	6.90	7.37	6.56	8.27	7.33
17	1 week 5 days	5.45	4.64	6.53	4.93	6.94	5.70
24	2 weeks 5 days	9.06	5.79	8.30	6.77	8.83	7.75
May 1	3 weeks 5 days	8.81	7.11	12.06	8.67	11.47	9.62
8	4 weeks 5 days	10.56	11.33	12.44	10.37	12.04	11.35
15	5 weeks 5 days	12.64	12.19	10.44	11.33	11.58	11.64
22	6 weeks 5 days	12.99	11.65	9.41	10.96	10.79	11.16

ERYTHROCYTES (MILLIONS PER CUBIC MILLIMETER OF BLOOD)

Apr. 5	6 to 10 hours	6.36	6.40	5.87	6.07		6.17
8	3 days	5.37	3.84	4.66	3.81	6.18	4.77
24	2 weeks 5 days		3.63	5.41	4.48	6.15	4.92
May 22	6 weeks 5 days	9.21	9.77			7.29	8.75

WEIGHTS OF PIGS (POUNDS)

Apr. 5	At birth	3.0	3.2	2.8	2.6	3.0	2.92
May 22	6 weeks 5 days	30.5	31.5		28.0	21.7	27.94

* Blood of sow 90 DJ contained 9.38 and 9.76 gm. hemoglobin per 100 c. c. when tested on Apr. 3 and Apr. 10, respectively.

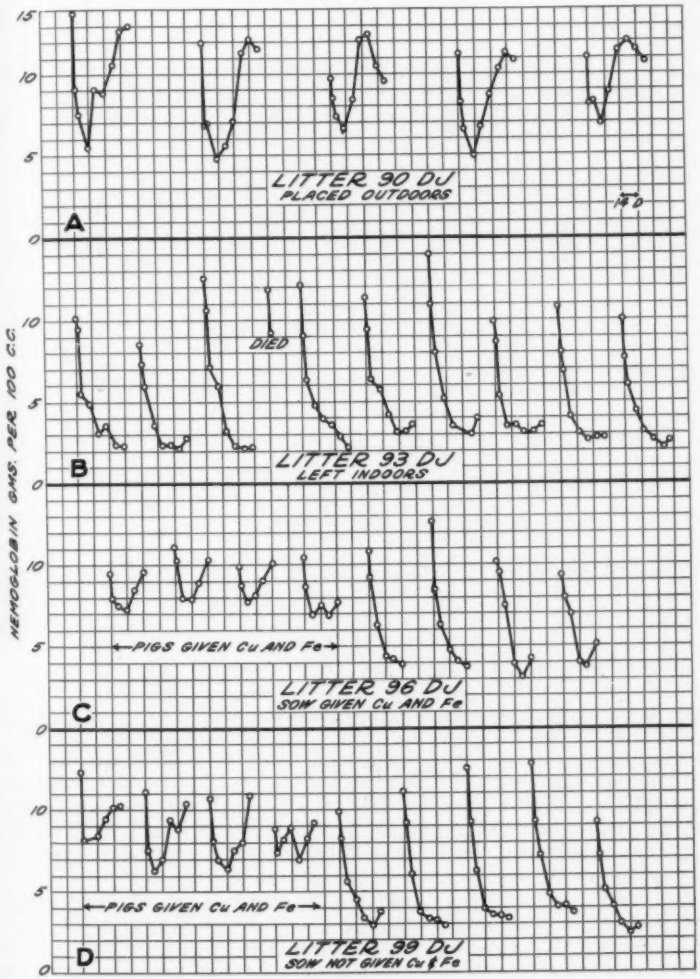

FIGURE 1.—The effect on the concentration of blood hemoglobin of suckling pigs of (A, B) outdoor as compared with indoor confinement; C, D, administration of iron and copper salts to the dam during gestation and lactation, and administration of iron and copper salts to the pigs themselves

TABLE 5.—*Blood hemoglobin and erythrocyte count, and weights, of a litter of pigs kept indoors*

[Litter 93 DJ*; sow and pigs kept inside; no medication]

HEMOGLOBIN (GRAMS PER 100 C. C. OF BLOOD)

Date	Age of pigs	Pig No. 93-90-B	93-9-B	93-90-B	93-30-B	93-30-B	93-60-B	93-90-S	93-9-S	93-3-S	93-30-S	Average
1929												
Apr. 13	2 hours	10.19	8.50	12.56	11.83	12.15	11.39	13.92	9.89	10.84	10.03	11.13
15	1 day	9.45	7.35	10.47	9.14	9.00	9.40	10.92	8.68	7.94	7.73	9.01
17	4 days	5.43	5.83	7.01	.....	6.28	6.31	7.94	5.31	6.96	5.97	6.34
24	1 week 4 days	4.99	3.57	5.92	.....	4.72	5.68	5.07	3.46	4.11	4.35	4.65
May 1	2 weeks 4 days	3.07	2.11	3.18	.....	3.90	4.12	3.43	3.47	3.05	3.16	3.28
8	3 weeks 4 days	3.44	2.17	2.21	.....	3.58	3.06	.....	3.04	2.62	2.63	2.84
15	4 weeks 4 days	2.35	2.04	2.06	.....	2.82	3.08	2.97	3.07	2.76	2.19	2.39
22	5 weeks and 4 days	2.27	2.79	2.19	.....	2.09	3.53	3.97	3.51	2.75	2.55	2.85
	ERYTHROCYTES (MILLIONS PER CUBIC MILLIMETER OF BLOOD)											
May 1	2 weeks 4 days	.....	2.28	.....	.....	4.98	.....	4.83	2.87	3.40	2.81	3.53
	WEIGHTS OF PIGS (POUNDS)											
Apr. 13	At birth	2.3	2.4	2.7	2.4	2.6	2.5	2.5	2.2	2.5	2.4	2.45
May 22	5 weeks 4 days	7.2	8.0	13.0	.....	12.2	15.5	17.0	14.0	15.5	12.0	12.71

* Blood of sow 93 DJ contained 11.67 and 11.58 gm. hemoglobin per 100 c. c. when tested on Apr. 3 and Apr. 13, respectively.

The first blood samples, taken from 2 to 10 hours after farrowing, indicate that the pig is born with a fairly high level of hemoglobin, averaging 11 to 11.5 gm. per 100 c. c. A rapid decrease occurs during the first week to a level but little more than half that at birth, and continues for four or five weeks if the pigs are left indoors (litter 93 DJ). Removal to an outside pen, with no access to soil or vegetation, brings about a prompt recovery (litter 90 DJ), both in blood picture and in physical condition. At 39 days of age the litter indoors averaged 17.71 pounds in weight, while at 47 days of age the outdoor litter averaged 27.94 pounds. While part of this difference in weight was due to age and part to a difference in size of litter, there can be little doubt that much of it was the result of the favorable influence of outside conditions. These two litters as they appeared at this time (May 29) are shown in Figure 2.

In order to obtain more evidence concerning the effect of copper and iron medication of suckling pigs on the concentration of hemoglobin in the blood and to determine whether this effect can be secured by treatment of the sow with copper and iron salts preceding parturition and during the suckling period, two litter-mate Duroc-Jersey sows, 96 DJ and 99 DJ, were bred to the same boar. Two weeks before farrowing time sow 96 DJ was given daily doses of ferric citrate (4.0 gm.) and copper sulphate (0.75 gm.) equivalent to 750 mgm. of iron and 191 mgm. of copper daily. The former as a solid and the latter dissolved in 50 c. c. of distilled water, were mixed with the feed. This dose was continued until the litter, farrowed on April 16, 1929, was removed from observation. Sow 99 DJ

received no iron or copper supplements at any time. Blood samples were taken from the individual pigs from two to five hours after birth, after which each litter was divided into two groups, containing four or five pigs each. One group in each litter received daily by mouth water solutions of ferric citrate and copper sulphate in amounts

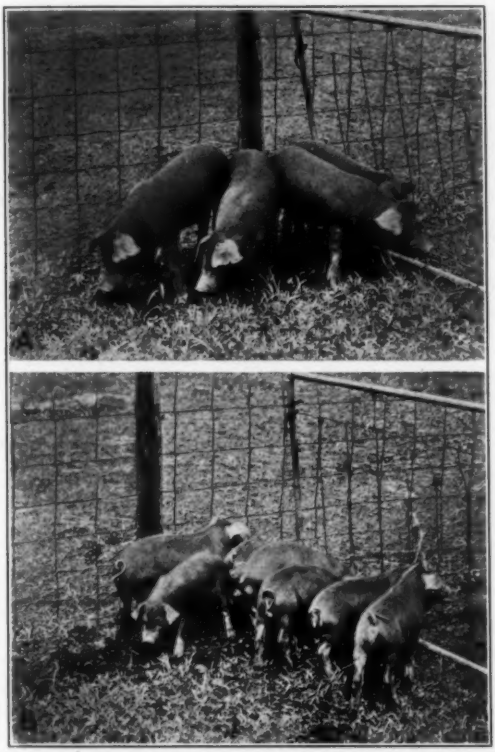

FIGURE 2.—The effect of indoor as compared with outdoor living conditions on the health and vigor of suckling pigs. The four pigs shown at A farrowed in the barn and were removed outdoors to cindered pens in about two weeks; their average weight was about 30 pounds each at the time the photograph was taken. The seven pigs shown at B farrowed in the barn and were kept there until a few days before this photograph was taken, at which time they were almost 2 months old. Long confinement indoors has reduced them to a distinctly anemic condition and at the time the photograph was taken their average weight was only about 14 pounds each. Although litter B was larger than litter A and eight days younger, the contrast in their size and condition must have been caused mainly by the difference between indoor and outdoor conditions of living

containing, respectively, 25 mgm. of iron and 5 mgm. of copper. During the observation of these two litters the little pigs were kept from the dam's feed and feces, either by putting them with the sow only at suckling time (every two or four hours) or, later, by cleaning the pen containing both sow and pigs every two or three hours and feeding the sow in another pen.

TABLE 6.—*Blood hemoglobin, erythrocyte count, and body weight of a litter of pigs half of which was given iron citrate and copper sulphate in individual doses; the sow had also been fed these salts*

[Litter 96 DJ[a]]

HEMOGLOBIN (GRAMS PER 100 C. C. OF BLOOD)

Date	Age of pigs	Pigs fed ferric citrate and copper sulphate				Control pigs				Average treated	Average controls
		96-99-S	96-9-S	96-90-B	96-9-B	96-30-B	96-39-S	96-30-S	96-90-S		
1929											
April 16	5 hours	9.38	11.16	9.82	10.42	10.79	12.61	10.19	9.34	10.19	10.73
17	30 hours	7.99	10.22	8.73	8.74	9.16	8.41	9.53	7.99	8.92	8.77
24	1 week, 1 day	7.42	7.91	7.68	6.88	6.29	6.28	7.46	6.90	7.47	6.73
May 1	2 weeks 1 day	7.24	7.90	8.06	7.58	4.37	4.71	3.99	4.00	7.69	4.27
8	3 weeks 1 day	8.41	8.82	9.00	6.93	4.24	4.01	3.12	3.78	8.29	3.79
15	4 weeks 1 day	9.56	10.33	10.11	[b]7.63	3.97	3.77	4.28	5.05	9.41	4.27

ERYTHROCYTES (MILLIONS PER CUBIC MILLIMETER OF BLOOD)

April 16	5 hours	6.67	8.07	6.73	5.71	7.09	8.71	7.09	5.76	6.80	7.16
May 8	3 weeks 1 day	6.84	7.43	8.73	5.29	3.76	3.59	4.88	4.13	7.07	4.09

WEIGHTS OF PIGS (POUNDS)

Apr. 16	At birth	2.5	2.5	2.6	2.6	2.5	2.4	2.8	2.5	2.55	2.55
May 8	3 weeks 1 day	9.5	8.5	10.0	11.0	7.5	8.0	11.0	9.0	9.75	8.87
15	4 weeks 1 day	9.0	8.5	10.0	10.5	8.5	10.0	14.0	9.0	9.50	10.37

[a] Blood of sow 96 DJ contained 12.06, 11.43, and 9.59 gm. hemoglobin per 100 c. c. when tested on Apr. 3, Apr. 15, and Apr. 17, respectively.
[b] Died May 16 of unknown cause.

TABLE 7.—*Blood hemoglobin, erythrocyte count, and body weight of a litter of pigs a part of which was given iron citrate and copper sulphate in individual doses, but when the sow had not been fed these salts*

[Litter 99 DJ[a]]

HEMOGLOBIN (GRAMS PER C. C. OF BLOOD)

Date	Age of pigs	Pigs fed ferric citrate and copper sulphate				Control pigs					Average treated	Average controls
		99-9-B	99-3-B	99-99-S	99-9-S	99-33-S	99-30-B	99-30-S	99-3-S	99-33-B		
1929												
Apr. 17	2 hours	12.39	11.14	10.61	8.89	9.86	11.10	12.48	12.73	9.24	10.76	11.08
18	40 hours	8.18	7.54	8.09	7.25	8.21	9.17	9.31	9.31	7.27	7.76	8.65
24	1 week	8.26	6.26	6.89	8.14	5.54	6.00	6.32	7.24	5.67	7.39	6.03
May 1	2 weeks	8.47	6.86	6.38	8.91	4.46	3.71	3.94	4.97	4.09	7.65	4.23
8	3 weeks	9.44	9.32	7.52	6.89	3.38	3.35	3.58	4.05	2.88	8.29	3.45
15	4 weeks	10.11	8.81	7.99	8.23	2.90	3.24	3.46	4.13	2.44	8.78	3.23
22	5 weeks	10.32	10.32	10.81	9.22	3.77	2.91	3.38	3.77	2.77	10.17	3.22

ERYTHROCYTES (MILLIONS PER CUBIC MILLIMETER OF BLOOD)

Apr. 17	2 hours	7.33	6.83	7.53	6.92	6.21	7.65	8.44	7.85	5.93	7.15	7.22
May 15	4 weeks	5.95	------	7.62	7.96	3.18	3.44	4.06	4.80	3.67	7.18	3.83

WEIGHTS OF PIGS (POUNDS)

Apr. 17	At birth	2.0	2.3	2.4	2.6	2.0	2.6	2.1	2.5	2.5	2.32	2.31
May 8	3 weeks	8.0	10.0	9.0	10.0	8.0	12.0	9.0	10.5	10.0	9.25	9.90
15	4 weeks	9.0	11.5	11.0	12.0	10.0	13.0	9.5	14.0	11.5	10.87	11.69
22	5 weeks	10.0	12.7	10.2	12.7	10.0	12.7	10.0	15.5	13.0	11.40	12.23

[a] Blood of sow 99 DJ contained 11.33 and 12.19 gm. hemoglobin per 100 c. c. when tested on Apr. 3 and Apr. 15, respectively.

All of the observations taken on these two litters are recorded in Tables 6 and 7. The hemoglobin results are shown graphically in Figure 1, C and D. The conclusions that may be drawn from these results seem clear cut. In both litters all of the pigs receiving copper and iron medication escaped anemic levels of hemoglobin. Although this treatment did not prevent a marked initial fall in hemoglobin concentration, in no case was a level of 6 gm. per 100 c. c. attained. For all but one pig (9–B in litter 96 DJ) a rapid recovery in hemoglobin occurred, so that at 4 or 5 weeks of age the birth level was again reached. The untreated pigs present an entirely different picture. The drop in hemoglobin concentration continued in every case until a level of 3 or 4 gm. or less per 100 c. c. was reached. It is noteworthy that the untreated pigs nursed by the sow receiving copper and iron medication were not appreciably benefited as judged by comparison with the pigs in the other litter. The effectiveness of the copper and iron treatment of suckling pigs in the prevention of nutritional anemia is clearly evident, as is the ineffectiveness of oral administration of these metals to the lactating sows.

It is interesting to note from Tables 6 and 7 that the body weights of the pigs were not favorably influenced by the copper and iron medication. Evidently the rigid restriction of the pigs to the milk of the dam prevented normal gains in weight.

The individual dosing of anemic pigs under practical farm conditions would of course be out of the question. It is unfortunate that supplementing of the sow's ration with salts of iron and copper—a procedure that would be thoroughly practical—is ineffective in preventing anemia in pigs. In all probability this is due to the fact that the iron and copper content of milk is not readily, if at all, raised by administering iron and copper salts to the lactating female (*5*, *6*).[6] Another possible method of dosing the pigs was to spread a solution of the metallic salts over the udder of the sow several times a day, so that the little pigs would of necessity remove some copper and iron salts from the teats while suckling.

This expedient was tried in the case of a litter of nine Hampshire pigs, No. 23 Hamp. Two solutions were made up, the first containing 7.86 gm. of $CuSO_4.5H_2O$ per liter and the second 42.8 gm. of ferric citrate per liter. These solutions were applied by hand to the udder of the sow four, five, or more times daily. Starting on June 27 a mixed solution of the salts containing corn sirup in addition was substituted. This solution was made by dissolving 15.72 gm. of hydrated copper sulphate in 500 c. c. of water; the solution was brought to the boiling point, and 85.6 gm. of powdered ferric citrate was added slowly with shaking. To the mixture was then added about 1 pint of corn sirup. This was applied to the udder of the sow by hand. The sow and her litter were kept indoors throughout the experimental period. The observations on this litter comprise Table 8. The hemoglobin results are given graphically in Figure 3, A.

[6] In unpublished experiments in cooperation with W. B. Nevens, of the Department of Dairy Husbandry, it has been found that daily doses of 3.2 gm. of hydrated copper sulphate given to dairy cows do not appreciably alter the copper content of milk. For a brief description of these experiments, which will be continued and extended to iron salts during the coming year, see *15*, *p. 120–121*.

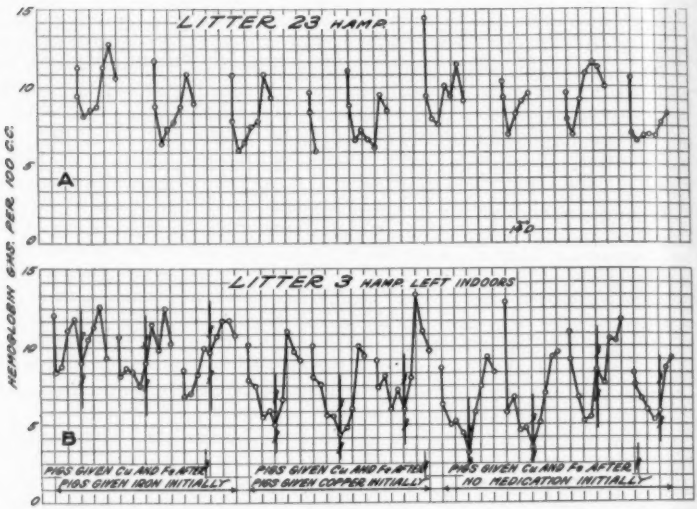
LITTER 23 HAMP.
A
LITTER 3 HAMP. LEFT INDOORS
B
PIGS GIVEN Cu AND Fe AFTER PIGS GIVEN IRON INITIALLY
PIGS GIVEN Cu AND Fe AFTER PIGS GIVEN COPPER INITIALLY
PIGS GIVEN Cu AND Fe AFTER NO MEDICATION INITIALLY
HEMOGLOBIN GMS. PER 100 C.C.
15
10
5
0

FIGURE 3.—The effect on the blood hemoglobin concentration of suckling pigs of (A) smearing a solution of iron and copper salts on the udder of the dam, and (B) of the separate and combined medication of the pigs with copper and iron salts

TABLE 8.—*Blood hemoglobin, erythrocyte count, and weights of a litter of pigs when the sow's udder was covered with a solution of iron citrate and copper sulphate*

[Litter 23 Hampshire; kept indoors]

HEMOGLOBIN (GRAMS PER 100 C. C. OF BLOOD)

Date	Age	Pig No. 23-30-B	23-9-B	23-90-B	23-99-B	23-30-S	23-9-S	23-90-S	23-99-S	23-60-S	Average
1929											
June 4	9 hours	11.26	11.74	10.73	9.66	11.06	14.40	10.33	9.62	10.51	11.03
5	1 day	9.45	8.72	7.73	8.36	8.59	9.38	9.27	7.82	6.92	8.47
12	1 week 1 day	8.10	6.22	5.87	5.87	6.50	7.92	6.89	6.86	6.50	6.75
19	2 weeks 1 day	8.53	7.35	6.43	(a)	7.17	7.60	8.18	9.12	6.82	7.65
26	3 weeks 1 day	8.74	7.78	7.38	------	6.55	10.10	9.02	10.78	6.87	8.40
July 3	4 weeks 1 day	11.32	8.65	7.81	------	6.03	9.32	9.50	11.57	6.81	8.88
10	5 weeks 1 day	12.62	10.99	10.76	------	9.44	11.48	------	11.23	7.66	10.60
15	6 weeks 1 day	10.68	8.89	9.22	------	8.50	9.10	------	9.93	8.27	9.23

ERYTHROCYTES (MILLIONS PER CUBIC MILLIMETER OF BLOOD)

June 4	9 hours	6.84	7.34	7.08	6.23	6.25	7.55	5.51	5.44	4.69	------
12	1 week 1 day	------	------	------	------	------	------	------	------	3.95	------

WEIGHTS OF PIGS (POUNDS)

June 4	At birth	3.5	3.4	3.1	3.1	3.4	4.0	3.5	3.0	3.1	------
5	1 day	4.0	4.1	3.9	3.2	4.0	4.5	4.0	3.5	3.9	------
12	1 week 1 day	6.5	6.5	6.5	6.0	7.0	7.5	7.0	6.0	6.5	------
19	2 weeks 1 day	9.5	9.5	10.0	(b)	11.0	11.0	11.0	8.5	11.5	------
26	3 weeks 1 day	10.5	12.0	14.0	------	13.5	13.5	14.0	10.0	13.0	------
July 3	4 weeks 1 day	12.0	14.0	17.0	------	16.0	16.0	18.0	13.5	16.0	------
10	5 weeks 1 day	14.0	19.0	18.5	------	19.5	20.5	------	16.0	18.5	------
15	6 weeks 1 day	16.5	24.0	24.5	------	24.0	26.0	------	21.0	23.5	------

[a] Dead. [b] Killed by sow.

The pigs in this litter increased in body weight at a rate that was not far from normal. The hemoglobin concentration of the blood decreased, as usual, for the first week or so, after which it increased

up to the fifth week of age, sometimes to a value higher than that initially observed. A comparison of the hemoglobin curves of this litter with those of litter 93 DJ (fig. 1, B) seems to indicate clearly that the method of administering copper and iron salts to suckling pigs by spreading a solution of the salts over the udder of the dam is an effective as well as a practical method of preventing milk anemia when, because of inclement weather, the pigs must be kept indoors. The experiment unfortunately did not continue long enough to evaluate the significance of the decrease in hemoglobin concentration, generally slight, that occurred in six of the eight surviving pigs from the fifth to the sixth weeks of age.

In the last experiment a litter of 10 Hampshire pigs was divided into three groups in order to determine the effect on the concentration of hemoglobin of the separate administration of ferric citrate and copper sulphate. Three of the pigs received ferric citrate in the ordinary dosage (equivalent to 25 mgm. of iron daily) three received copper sulphate (equivalent to 5 mgm. of copper daily) and four were untreated. At 4 weeks of age all pigs received the combined treatment of iron and copper. The experiment is not particularly conclusive, except in showing again the markedly favorable effect of the combined dosage. The hemoglobin changes are presented in Figure 3, B. All of the pigs receiving iron showed the initial drop in hemoglobin, but all of them showed some response to the iron medication. In two cases the response was marked. The sample of ferric citrate used contained 0.73 mgm.[7] of copper per gram, so that in this group each pig received 0.01 mgm. of copper daily in its dose of ferric citrate. The three pigs receiving copper sulphate only were not benefited appreciably by this treatment. All pigs responded promptly to the combined dosage of iron and copper.

SUMMARY

A few hours after birth the hemoglobin concentration in the blood of pigs was found to range from less than 9 to almost 15 gm. per 100 c. c., averaging 10.75 gm. in the 54 pigs tested. There is a rapid decrease in hemoglobin during the first few days, a decrease which starts at or soon after birth.

In the case of litters remaining indoors, this decrease in the level of hemoglobin in the blood continued until concentrations of 4, 3, or even 2 gm. per 100 c. c. of blood were reached. In the case of litters removed outdoors to cindered pens, this decrease was stopped and an increase was induced until the birth level of hemoglobin was again reached.

The birth level of hemoglobin could be restored also by administering ferric citrate and copper sulphate to the pigs, either by pipette in daily doses equivalent to 25 mgm. of iron and 5 mgm. of copper, or by spreading a solution of these metallic salts over the udder of the lactating sow. However, the administration of copper and iron salts to the dam during the last two weeks of gestation and the period of lactation had no appreciable effect on the blood hemoglobin concentration of the suckling pigs.

The administration of copper sulphate alone to the suckling pigs was ineffective in promoting the regeneration of hemoglobin. Ferric citrate alone, contaminated with a very small amount of copper, was

[7] Determined by the potassium ethyl xanthate method.

appreciably effective, but much less so than a combination of copper sulphate and ferric citrate.

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LINKAGE STUDIES WITH "SLASHED" AND "GLOSSY$_1$" OF THE "BN" LINKAGE GROUP IN MAIZE[1]

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INTRODUCTION

Maize furnishes unusually satisfactory material for the study of linkage relations in plants. Recessive characters which can be differentiated in the seedling state are especially valuable in linkage studies. The character "slashed," reported in this paper, was first observed in seedling progenies of three selfed ears of a 3-year selfed strain of Minnesota No. 13 yellow dent corn. These progenies were grown in the greenhouse during the winter of 1922–23. Apparently the same character has appeared in a number of unrelated selfed strains of Minnesota No. 13 since that time. Lindstrom (*7*)[2] reported a character which he described as "slashed or shredded seedling," and R. A. Emerson provided the writer with material described as "slashed." In the studies conducted at University Farm, St. Paul, there did not appear to be any resemblance between the type obtained from Emerson and the slashed seedling character reported in this paper.

It was early learned that the character slashed was linked with a glossy character obtained from Rustler white dent, which was found to be "glossy$_1$" of the "*Bn*" group. Crosses were made for the purpose of supplementing the available data (*6*) concerning the independence of the *Bn* group from other linkage groups. Data on the inheritance of the slashed character and linkage studies of slashed and "glossy$_1$" in relation to characters in the established linkage groups are presented in this paper.

DESCRIPTION OF SLASHED

Slashed is a leaf character. Its appearance is characterized by long, thin, chlorophyll-deficient striae on each leaf of the plant, usually in greater abundance near the tip. (Fig. 1.) These thin spots break through in later stages of plant development and give the leaf a slashed appearance. Slashed plants are usually much less vigorous than normal plants (fig. 2) and generally somewhat later in maturing. In a favorable season small ears may be obtained from most slashed plants. The character is generally very definite and easy to distinguish at any stage from the seedling to the mature plant. In a very few of the crosses studied, however, slashed plants were almost as vigorous as normal ones, and classification was more difficult. This was particularly true of segregates from the tunicate versus slashed cross.

[1] Received for publication May 25, 1929; issued May, 1930. Published with the approval of the director as Journal Series Paper No. 854, Department of Agriculture, University of Minnesota.

[2] Reference is made by number (italic) to "Literature cited," p. 950.

INHERITANCE OF SLASHED

Data for the segregation of normal and slashed plants in several selfed lines of Minnesota No. 13 are summarized in Table 1.

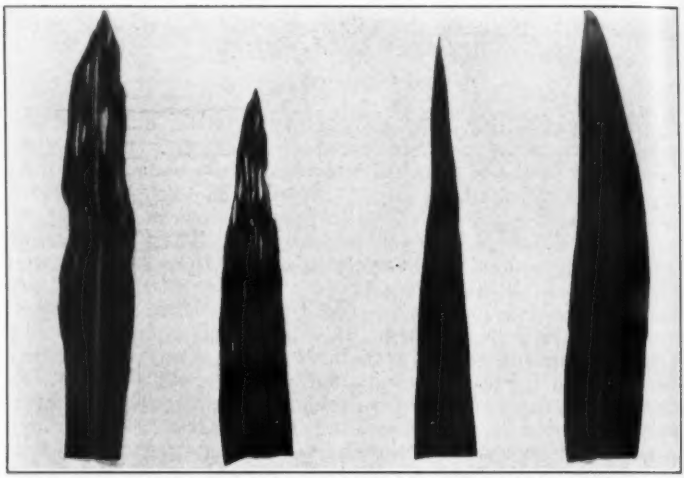

FIGURE 1.—The first two seedling leaves of slashed (left) and normal (right) maize plants

TABLE 1.—*Segregation for slashed plants in Minnesota No. 13 selfed strains*

Strain No.	Years selfed	Number of plants	
		Normal	Slashed
42-2	3	17	3
42-4	3	64	20
42-5	3	61	27
9-1	1	61	20
1-8	1	15	4
1-32	1	121	39
30-1	3	303	102
30-2	3	15	4
Total		657	219
Expected for 3 : 1 segregation		657	219

There were 657 normal plants and 219 slashed, which agrees perfectly with a 3:1 ratio. This indicates that slashed is a simple recessive character. In the F_2 generations of certain crosses made for the purpose of determining the linkage relations of slashed, however, the obtained segregation approached a 13:3 ratio, which suggested the possibility of two factor pairs. One might assume the complementary action of a recessive factor with a dominant factor necessary for the production of slashed plants. With both factor pairs segregating, 2 of every 3 slashed F_2 plants when selfed would be expected to segregate in the ratio of 3 slashed to 1 normal. A large number of self-pollinations were made on slashed plants in culture A4a, an F_2 generation which segregated in approximately a 13:3 ratio. (Tables 2 and 4.) Selfed ears were obtained on 24 plants, although the number of seed obtained was rather small in some cases. The data for the F_2 segregation and for the F_3 progenies of selfed F_2 ears are presented in Table 2.

FIGURE 2.—Slashed maize plant (left) and normal plant (right) from a segregating line just before silking

TABLE 2.—*Breeding behavior of normal and slashed plants in the F_2 generation, and in the F_3 progenies of selfed slashed plants*

Description		Normal	Slashed
F_2 culture A4a	Obtained	1,863	421
	Expected 13: 3	1,856	428
F_3 progenies	18 lines		(a)
	2 lines	(b)	(b)
	1 line	1	41
	----do	3	32
	----do	2	5
	----do	2	3

[a] All. [b] Too weak to classify.

Only 4 of the 24 selfed ears obtained from F_2 slashed plants produced progeny which contained any normal seedlings, while 2 of these gave too few normals to be considered as probable 3: 1 ratios. From

the F_3 line which segregated for 2 normal and 3 slashed plants, 1 normal plant was selfed. On greenhouse test, the progeny of this plant consisted of 75 normal and 20 slashed plants, a deviation of 4 ± 2.5 from a 3:1 ratio. It is evident that this normal plant carried a recessive factor for slashed, which indicates that it resulted from accidental cross-pollination. In case two factors were necessary for the expression of slashed in culture A4a, one would expect the progeny of 16 slashed plants out of the 24 to segregate in a ratio of 3 slashed to 1 normal. It appears obvious that the few normal seedlings obtained were the result of off-pollination, and that the deviation from a 3:1 ratio obtained in culture A4a can not be explained by a factorial hypothesis which assumes the interaction of a dominant and a recessive factor necessary for the expression of slashed. In culture A4a (see summary of F_2 data, Table 4) and in several other crosses reported in this paper, the percentage of slashed seedlings is less than expected on the basis of a 3:1 ratio of normal to slashed, this being, apparently, for the most part, due to the deficiency of slashed in the double recessive class. From a careful study of all of the data, it is concluded that slashed is a simple recessive character, and the factor pair for normal versus slashed is designated *Slsl*.

STUDIES OF SLASHED IN RELATION TO FACTORS IN KNOWN LINKAGE GROUPS

Slashed has been studied in relation to one or more factors in each of the eight known linkage groups. The linkage value in most cases was calculated in terms of p, or the crossover value expressed as a decimal fraction, which was obtained by the use of Yule's coefficient of association. (See Collins (*1*).) Probable errors for p were computed from tables prepared by F. R. Immer of this division.

An alphabetic list of the factors which were studied in relation to *sl* is given below. References to publications for the characters listed may be found in Hayes and Garber (*4*). The factor symbols for dominant characters are capitalized.

A. Anthocyan, general plant color found in aleurone, pericarp, stems, leaves, etc.
br. Brachytic, characterized by shortened internodes.
Bn. Brown aleurone.
C. Colored aleurone, complementary to *A* and *R*.
Fl. Flinty endosperm, *fl* floury.
g. Golden plant color.
gl. Glossy leaf.
lg. Liguleless leaf.
P. Pericarp and cob color. An allelomorphic series P^{rr}, P^{wr}, etc.
Pr. Purple aleurone.
R. Colored aleurone, complementary to *A* and *C*.
ra. Ramose ear.
sh. Shrunken endosperm.
su. Sugary endosperm.
ts_1, ts_2. Tassel seed. Produces only pistillate flowers in the tassel.
Tu. Tunicate ear.
wx. Waxy endosperm.
Y. Yellow endosperm.

The value of p for obtained F_2 ratios involving the complementary factors *A*, *C*, and *R* in relation to *sl* was calculated from formulae presented by Owen (*8*). By this method, the degree of association in inheritance is expressed by means of a product moment coefficient of

correlation r; and the p or crossover value is calculated from r for various phenotypic ratios. The summarized F_2 data for these studies are presented in Table 3.

TABLE 3.—*Summary of F_2 data for crosses of sl with the A, C, and R aleurone factors*

Culture No.	Colored		Colorless		Total	Per cent slashed		r	Factor relations	p
	Sl	*sl*	*Sl*	*sl*		Colored	Colorless			
H 46	603	161	824	249	1,837	21.1	23.2	0.025±0.016	{Coupling *C* or *R*..	0.463
H 175	1,022	258	896	287	2,463	20.2	24.3	.049± .014	{Repulsion *A*..... Coupling *C* or *R*..	.537 .446
						r/*P. E.*		H 46=1.6; H 175=3.5.		

In each cross, the colored seeds produced a smaller percentage of slashed plants than did the colorless. In view of the fact that the coupling phase for the C and R factors in relation to *sl* was studied in each case, the data suggest the possibility of a weak linkage. The apparent association, however, is very slight, as indicated by a correlation coefficient of 0.025 ± 0.016 for H 46 and 0.049 ± 0.014 for H 175. In no case were more than 25 per cent slashed plants obtained from the colorless seeds, and when considered in relation to other linkage data in the A, C, and R, linkage groups, it seems probable that *sl* is inherited independently of A, C, and R.

LINKAGE STUDIES WITH THE C GROUP

The repulsion phase involving F_2 segregation for *sl* in relation to *sh* and *wx* was studied. The summarized data are presented in Table 4.

TABLE 4.—*Summary of F_2 data for crosses of sl with sh and wx of the C group*

Culture No.	*Sh*		*sh*		Total	Per cent slashed		p
	Sl	*sl*	*Sl*	*sl*		*Sh*	*sh*	
I_1 175	1,457	424	461	121	2,463	22.5	20.8	
H 173	265	82	61	18	426	23.6	22.8	
Total	1,722	506	522	139	2,889			0.487±0.010
	Wx		*wx*			*Wx*	*wx*	
A 4a	1,434	364	429	57	2,284	20.2	11.7	.409±.012
H 176A	1,157	366	349	102	1,974	24.0	22.6	.489±.012
Total	2,591	730	778	159	4,258			.455±.008

With the exception of culture A 4a, the data indicate independent inheritance of *sl* with the *sh* and *wx* factors. In culture A 4a, there is a deficiency of slashed for a 3 : 1 ratio of normal to slashed, the deficiency being chiefly in the double recessive class. The deviation of p from 0.50 for independent inheritance is 2.3 times the probable error for the *sl-sh* crosses and 5.6 times the probable error for the total data for the *sl-wx* crosses. This rather large deviation from the

expected in the latter case appears to be principally due to the deficiency in the double recessive class in cross A 4a, the p value of 0.489 ± 0.012 for H 176A clearly indicating independent inheritance for the factors *wx* with *sl*. When the data for these crosses are considered in relation to those presented for the *A*, *C*, and *R* factors, it appears very probable that *sl* is independent in inheritance of *C*, *sh*, and *wx* factors. The relation of *wx* and *sl* will, however, be studied further in back-cross material.

LINKAGE STUDIES WITH THE R GROUP

The relation of *sl* with *g* was studied in the repulsion phase in one cross. A summary of the data obtained is presented in Table 5.

TABLE 5.—*Summary of F_2 data for a cross of sl with g of the R group*

Culture No.	*G*		*g*		Total	Per cent slashed		p
	Sl	*sl*	*Sl*	*sl*		*G*	*g*	
H 176B	948	301	290	45	1,584	24.1	13.4	0.400±0.014

There appears to have been considerable elimination of double recessives since only 13.4 per cent of the golden plants were slashed, whereas the normal green plants segregated in approximately a 3:1 ratio of nonslashed to slashed. When considered in relation to the data presented for the *A*, *C*, and *R* factors it is apparent that *sl* is probably independent of the *R* and *g* factors in this group. The *g*-*sl* relation will be further tested, however, in back-cross material.

LINKAGE STUDIES WITH THE SU GROUP

F_2 generations of four crosses involving segregation for *su* and *sl* in the repulsion phase were studied. The origin of the *su* factor was different in three crosses. Intercrosses of these three sugary parents were made, and only sugary kernels were obtained in each case, indicating only one *su* factor concerned in all crosses. The relation of *Tu* and *sl* was studied in the progeny of tunicate plants obtained from a cross of *Tutu Slsl*×*tutu slsl*. A summary of the data for these crosses in presented in Table 6.

TABLE 6.—*Summary of F_2 data for crosses of sl with su and Tu of the su group*

Culture No.	*Su*		*su*		Total	Per cent slashed		p
	Sl	*sl*	*Sl*	*sl*		*Su*	*su*	
A 9a and b	1,498	473	321	33	2,325	24.0	9.3	0.345±0.012
A 11a and b	1,533	528	362	91	2,514	25.6	20.1	.456± .011
H 46	875	281	273	57	1,486	24.3	17.3	.440± .014
H 180	492	182	168	25	867	27.0	13.0	.373± .019
Total	4,398	1,464	1,464	206	7,192			.416± .006
	Tu		*tu*			*Tu*	*tu*	
A 9a&b	363	69	143	25	600	16.0	14.9	0.512±0.021

The p value for the sugary slashed crosses varies from 0.345 to 0.456 in direct relation to the deficiency of the double recessive class. The percentage germination for the four crosses, respectively, are *Su*, 95.0, 93.3, 99.1, and 100.0; and *su*, 49.3, 60.8, 86.4, and 61.3. The percentage of slashed plants obtained from starchy seeds in each cross approached 25.0, indicating a 3:1 ratio and independence of the factors *su* and *sl*. Too few slashed plants were obtained from both nontunicate and tunicate groups of culture A9a and A9b. This deficiency was similar in both groups and there appears to be independent inheritance of the factors *Tu* and *sl*. It is a fact of considerable interest that the slashed plants obtained from the tunicate-slashed cross were almost as vigorous as nonslashed plants.

LINKAGE STUDIES WITH THE B GROUP

A study was made of *sl* in relation to ts_1 and *lg* in F_2 material and of *sl* with *lg* in back-cross material (culture H188). The factors entered in the repulsion phase in each case. A summary of the data is presented in Table 7.

TABLE 7.—*Summary of F_2 data for sl with ts_1 and lg, and back-cross data with lg*

[The factors ts_1 and *lg* are members of the *B* group]

Culture No.	*Ts*$_1$		*ts*$_1$		Total	Per cent slashed		p
	Sl	*sl*	*Sl*	*sl*		*Ts*$_1$	*ts*$_1$	
H 178	677	156	156	52	1,041	18.7	25.0	0.552±0.015
	Lg		*lg*			*Lg*	*lg*	
A 2$_1$	381	111	129	41	662	22.6	24.1	.512± .019
H 188	97	92	112	105	406	48.7	48.4	.498± .017

The factors *lg* and ts_1 are located at opposite ends of the chromosome. When considered in relation to this fact, the results obtained indicate slashed to be independent of factors in the B group.

Possible linkage relations between flinty-floury endosperm and slashed were studied. In F_2 flinty-floury segregates in a 1:1 ratio. A linkage of the factor which differentiates flinty from floury with a glossy seedling factor gl_2 was reported by Hayes and Brewbaker (*4*). Flinty-floury and gl_2 were recently found linked with *lg*. The summarized data for an F_2 generation segregating for flinty versus floury and normal versus slashed are given in Table 8.

TABLE 8.—*Summary of F_2 data for a cross of slashed flinty with nonslashed floury*

Data for culture No. H173	Flinty		Floury		Total	Per cent slashed	
	Sl	*sl*	*Sl*	*sl*		Flinty	Floury
Observed	983	274	1,007	270	2,534	21.8	21.1
Calculated	987	270	1,003	274			
Difference	4	4	4	4			

χ^2=0.15; P=very large.

As a test for linkage χ^2 was used. The theoretical population was obtained from the observed ratio rather than from the calculated on the basis of a 1 : 1 segregation for flinty to floury and a 3 : 1 segregation for normal and slashed (Collins, (*1*)). The value of *P*, which was used as a means of testing the agreement of observed with a calculated ratio, assuming independent segregation, is very large, indicating a very good fit. In a previous paper (*4*), it was stated that flinty-floury was independent in inheritance of Gl_1gl_1 and *Rara*. As *sl* is linked with factors in the *Bn* linkage group, it should be inherited independently of the factor pair for flinty-floury.

LINKAGE STUDIES WITH THE Y GROUP

Slashed was studied in relation to the *Y* factor in F_2 generations of six crosses, the *Y* factor in each case coming from the slashed parent. The summarized data are presented in Table 9.

TABLE 9.—*Summary of F_2 data for crosses of sl with Y of the Y group*

Culture No.	*Y*		*y*		Total	Per cent slashed		*p*
	Sl	*sl*	*Sl*	*sl*		*Y*	*y*	
A 11a	591	193	207	59	1,050	24.6	22.2	
H 44	829	259	316	102	1,506	23.8	24.4	
A 9a	679	229	255	69	1,232	25.2	21.3	
H 176A	781	239	249	90	1,359	23.4	26.5	
H 176B	920	258	329	77	1,584	21.9	19.0	
H 173	1,268	333	434	143	2,178	20.8	24.8	
Total	5,068	1,511	1,790	540	8,909	23.0	23.2	0.504±0.009

The results obtained are similar for each of the crosses studied and indicate independent inheritance of the *sl* and *Y* factors.

LINKAGE STUDIES WITH THE P GROUP

Studies were made with F_2 generation material involving *sl* in the repulsion phase in relation to *br* and ts_2, and the coupling phase with *P* for cob color. The summarized data are presented in Table 10.

TABLE 10.—*Summary of F_2 data for crosses of sl with br, ts_2, and P of the P group*

Culture No.	*Br*		*br*		Total	Per cent slashed		*p*
	Sl	*sl*	*Sl*	*sl*		*Br*	*br*	
H 175	690	217	180	62	1,149	23.9	25.6	0.512±0.015
	Ts_2		ts_2			Ts_2	ts_2	
H 172	504	157	159	44	864	23.8	21.7	.483± .018
	P		*p*			*P*	*p*	
A 11a and b	752	130	275	42	1,199	14.7	13.2	.518± .015

These data indicate independent inheritance of the characters involved.

LINKAGE STUDIES WITH THE A GROUP

Segregation for the *A* factor, as indicated by seedling stem color, was studied in the repulsion phase in two crosses in relation to slashed. The results are summarized in Table 11.

TABLE 11.—*Summary of F_2 data for a cross of sl with A of the A group*

Culture No.	*A*		*a*		Total	Per cent slashed		*p*
	Sl	*sl*	*Sl*	*sl*		*A*	*a*	
H 44	820	246	268	100	1,434	23.1	27.2	0.530±0.013
H 46	817	223	610	184	1,834	21.4	23.2	.514± .012

Slashed appears to be independent in inheritance of the *Aa* factor pair.

LINKAGE RELATIONS WITH THE BN GROUP

Definite linkage values have been obtained with slashed and characters whose factor locations are in the *Bn* group. The factors gl_1, *ra*, and *Bn* were studied in relation to *sl*. It has been possible to study only F_2 generation material up to the present time. The characters entered in the repulsion phase in each cross. The summarized data are presented in Table 12.

TABLE 12.—*Summary of F_2 data for crosses of sl with gl_1, ra and Bn of the Bn group*

Culture No.	*Gl_1*		*gl_1*		Total	Per cent slashed		*p*
	Sl	*sl*	*Sl*	*sl*		*Gl_1*	*gl_1*	
A 5a	181	50	94	2	327			
H 173	1,332	546	709	16	2,603			
Total	1,513	596	803	18	2,930	28.3	2.2	0.163±0.012
	Ra		*ra*			*Ra*	*ra*	
H 174	1,127	492	510	22	2,151	30.4	4.1	.210± .014
	Bn		*bn*			*Bn*	*bn*	
H 44	214	102	102	0	418			
A 9a	185	69	70	1	325			
H 174	135	55	61	4	255			
H 180	454	202	207	5	868			
Total	988	428	440	10	1,866	30.2	2.2	.157± .015

Since there appears to have been considerable zytotic elimination in many of the slashed crosses, particularly in the double recessive groups, crossover values can be considered only as giving rough approximations of the location of the factors in the chromosome. Crossover percentages of 38.2 for *Bn* versus *ra*, and 18.7 and 29.4 for *Bn* versus gl_1 were reported by Kvakan (*6*). The crossover percentages of 16.3 ± 1.2 for *sl* versus gl_1, 21.0 ± 1.4 for *sl* versus *ra*, and 15.7 ± 1.5 for *sl* versus *Bn* were obtained by using Yule's coefficient of association method. When calculated by Haldane's method (*2*), the following crossover percentages were obtained: 16.2 for *sl* versus gl_1,

21.0 for *sl* versus *ra*, and 16.6 for *sl* versus *Bn*. These data indicate the order of the genes to be as follows: *ra*—*gl*$_1$—*sl*—*Bn*.

A further study of the relationship of *sl* with factors in this linkage group is being made in back-cross material in order to determine with greater accuracy the location of the *sl* factor.

LINKAGE STUDIES OF SLASHED WITH THE PR FACTOR

Segregation for the *Pr* aleurone factor was studied in the repulsion phase in relation to *sl* in three crosses, the summarized results being presented in Table 13.

TABLE 13.—*Summary of F*$_2$ *data for crosses involving segregation for sl and Pr*

Culture No.		Purple		Red		Total	Per cent slashed		*p*
		Sl	*sl*	*Sl*	*sl*		Purple	Red	
H 46	Observed	372	111	231	50	764	23.0	17.8	0.455±0.019
	Calculated	381	102	222	59				
	Difference	9	9	9	9	X^2=2.8	P=0.10		
H175	Observed	647	176	375	82	1,280	21.4	17.9	.469± .015
	Calculated	657	166	365	92				
	Difference	10	10	10	10	X^2=2.1	P=0.15		
A49	Observed	734	120	242	53	1,149	14.1	18.0	.541± .014
	Calculated	725	129	251	44				
	Difference	9	9	9	9	X^2=2.9	P=0.09		

In cultures H 46 and H 175, too many red kernels were obtained to approximate a 3:1 ratio, the observed ratios of purple to red being 1.71:1 and 1.79:1, respectively. These ratios are similar to those reported by Hayes and Brewbaker (*3*). The χ^2 goodness of fit method was used to test for independent inheritance. The *P* values for the three crosses are: 0.10, 0.15, and 0.09 respectively. It may be said that a worse fit could be expected on the basis of random sampling in from 9 to 15 out of 100 trials for these crosses. The crossover value was also calculated in terms of *p*. In no case was the deviation from 0.500 as great as three times the probable error. The results indicate that the *Pr* and *sl* factors are probably inherited independently.

LINKAGE STUDIES OF GL$_1$ WITH OTHER FACTORS

Linkage studies of a glossy character, obtained from a selfed line of Rustler white dent, were started in 1923. It was later learned from crosses with Brunson's *gl*$_1$ that the glossy factor in the Rustler strain was the same as *gl*$_1$. The linkage studies were completed and are reported here since they further verify the independence of the *Bn* group from other linkage groups. In all the studies made F_2 data were used, the linkage values being calculated by Yule's coefficient of association method (*1*) except those for cultures H 159 and H 160, where more than one factor segregated for aleurone color. For these crosses, Owen's coefficient of correlation method was used (*8*). The data for *gl*$_1$ in relation to the aleurone factors *Pr*, *A*, *C*, and *R* are summarized in Table 14.

TABLE 14.—*Summary of F_2 data for crosses of gl_1 with the aleurone factors A, C, R, and Pr*

Culture No.	Purple		Red		Total	r	r/P.E.	p	Factor relations
	Gl	*gl*	*Gl*	*gl*					
H 159	250	75	142	40	510			0.486±0.023	Repulsion *Pr*.
H 160	778	245	260	75	1,358			.487± .014	Repulsion *Pr*.

Culture No.	Colored		Colorless		Total	r	r/P.E.	p	Factor relations
	Gl	*gl*	*Gl*	*gl*					
H 159	392	118	522	175	1,207	0.023±0.019	1.2	*A*=0.533	Repulsion *A*.
H 160	1,038	320	936	291	2,585	.002± .013	0.1	*C&R*= .467	Coupling *C&R*.
								.408	Coupling *C&R*.

The value of p closely approaches 0.500 in each case, indicating independent inheritance of gl_1 and the aleurone factors *Pr*, *A*, *C*, and *R*.

The factor gl_1 was studied in relation to the factors *wx*, *sh*, *g*, *su*, *Tu*, *Y*, and *P*, representing five linkage groups. The data for these crosses are presented in Table 15.

TABLE 15.—*Summary of F_2 data for crosses of gl_1 with other factors in known linkage groups*

Culture No.	*Gl*₁		*gl*₁		Total	p	Factors studied	Phase[b]	Linkage group
	X[a]	*x*[a]	*X*[a]	*x*[a]					
14b	201	79	80	36	396			*R*	*C*
17a	178	42	60	17	297			*R*	
H 160	1,235	426	396	129	2,186			*R*	
H 161	405	156	157	52	770			*R*	
Total	2,019	703	693	234	3,649	0.497±0.008	*wx*		
H 165	209	90	109	44	512			*R*	
H 167	727	245	265	68	1,305			*R*	
Total	996	335	374	112	1,817	.483± .012	*sh*		
13a	808	242	258	73	1,381	.493± .014	*g*	*R*	*R*
10b, 10c, 17a	943	151	326	69	1,489			*R*	*su*
H 164	220	55	79	31	385			*R*	
Total	1,163	206	405	100	1,874	.543± .010	*su*		
17b	194	83	59	22	358	.519± .027	*Tu*	*C*	
H 173	954	385	365	126	1,830	.522± .012	*Y*	*C*	*Y*
13a	426	145	108	43	722	.478± .018	*P*[c]	*C*	*P*

[a] *X*=dominant; *x*=recessive. [b] *R*=repulsion; *C*=coupling. [c] *P*=Cob color.

The data indicate independence of gl_1 and other factors considered. In only one case does deviation from 0.50 exceed the probable error by more than twice its value. In the *su* versus gl_1 cross the deviation from 0.50 was 4.8 times as great as the probable error. There is a deficiency of numbers in both *su* classes. In crosses 10b, 10c, and 17a the germination of the sugary seeds was low, the relative percentage germination for starchy and sugary seeds being 81.9 and 42.6, respectively. In the cross H 164, there was a lack of sugary seeds

in the F_2 generation, the segregation being 322 starchy to 88 sugary. This is a deviation of 14.5 ± 5.9 from the expected 3:1 ratio. The germination percentage for starchy and sugary seeds in this cross was not greatly different, being 92.9 and 97.7, respectively.

SUMMARY AND CONCLUSIONS

A seedling and plant character known as "slashed" was obtained as a segregate from several selfed lines of Minnesota No. 13 yellow dent corn. In these lines, slashed appeared to be a simple recessive, a perfect fit of actual and expected having been obtained. In certain crosses studied later, far too few slashed plants were obtained for a single-factor difference. It was shown in one case, where the results obtained in F_2 closely approximated a 13:3 ratio, that a factorial hypothesis which would give such a ratio did not explain the results. In view of this, and the fact that in several crosses the double recessive class was greatly reduced, it is concluded that slashed is a simple recessive, and the factor pair concerned has been designated *Slsl*.

Segregating generations involving the factor for slashed in relation to factors in each of the eight well-known linkage groups, as well as *Prpr*, whose definite linkage relations are not known, have been studied for the most part in F_2 material. In all cases, except for factors in the *Bn* group, the data indicate probable independent inheritance.

The independence of *sl* and other members of the *Bn* group from other linkage groups is further verified by F_2 linkage studies of gl_1 in relation to factors in the *C*, *R*, *su*, *Y*, *P*, and *A* groups in which independent inheritance is indicated in every case.

Studies of the linkage relations of *sl* with gl_1, *ra*, and *Bn* of the *Bn* group were made with F_2 material, the following crossover values being obtained by Yule's coefficient of association method: *sl* versus gl_1, 16.3 ± 1.0; *sl* versus *ra*, 21.0 ± 1.4; and *sl* versus *Bn*, 15.7 ± 1.5. When considered in relation to the data reported by Kvakan (*6*), the order of the genes appears to be *ra*—gl_1—*sl*—*Bn*.

The results of these studies further support those of an earlier investigation in indicating that the *Bn* linkage group is independent of other groups.

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TOXICITY OF SULPHUR TO SPORES OF SCLEROTINIA CINEREA AS AFFECTED BY THE PRESENCE OF PENTATHIONIC AND OTHER SULPHUR ACIDS[1]

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INTRODUCTION

The studies on sulphur reported in the original paper by Young (*8*)[2] were summarized as follows: (1) Particulate sulphur is more toxic than the more coarsely ground sulphur; (2) the toxic factor is formed by the action of water and oxygen; (3) this toxic factor is formed only in the acid range and is an acid itself, pentathionic acid; (4) the gases of sulphur are not toxic in quantities present.

Since that work was published many changes have been made in commercial dust formulas. The alkaline type of fluffer has been largely discarded, and the sulphur dusts have been made finer by using a guaranteed 300-mesh instead of a 200-mesh formerly used. These new dusts are much more effective, and consequently the dust method of applying fungicides is gaining ground. The above work has also led to the development of some promising wettable sulphur sprays.

In spite of all these improvements sulphur dusts remain somewhat ineffective under some of the severe conditions for control. Either the dusts are not toxic enough, or they wash off. These problems remain unsolved, and until further progress is made a sulphur dust that will be universally effective for the control of certain fungous diseases at least must remain lacking. It was thought advisable therefore to continue the study of the toxic property of sulphur and especially to determine the effect of the various factors, such as reaction, oxidation, and general weathering, upon the yield of this fungicidal property.

Considerable discussion has arisen about the chemical nature of the exact toxic factor. A few papers have been published, some of which confirm, while others question, the toxicity of pentathionic acid. Tisdale (*6*) found that pentathionic acid is a toxic factor of sulphur. Lee and Martin (*4*), assuming that the toxic property of sulphur is an oxidation product, added an oxidizing agent and produced a sulphur dust which was many times more effective than sulphur alone. They have protected their product by a patent. Roach and Glynne (*5*) tested a great many acids and salts of sulphur and tentatively concluded that the toxicity of sulphur is due to thiosulphuric acid. An examination of their tables shows that pentathionic acid is quite toxic. They stated also that many of their solutions were cloudy, an indication that colloidal sulphur, and consequently pentathionic acid, were present. Young and Williams (*9*) confirmed the previous work by Young (*8*) and further showed that ordinary forms of sulphur have pentathionic acid associated with them.

An opposing view of the toxic factor of sulphur is held by Barker and his associates (*1*). He stated in his report that no pentathionic

[1]Received for publication July 19, 1929; issued May, 1930.
[2] Reference is made by number (italic) to "Literature cited," p. 962.

acid could be found on sulphur. He did not give the tests used, but probably did not use the same as were used in this laboratory, or he would have obtained a positive test. He attributed the toxic action of sulphur to particulate sulphur. Later, in his annual report (*2*), he concluded that the toxic action is due to hydrogen sulphide, this being formed by the action of the leaf on the sulphur particle.

The aim of the sulphur work carried on in this laboratory during the past few years has been to develop an effective dusting sulphur. The work has been divided into three parts: (1) The chemistry of sulphur and its toxic factor or factors; (2) toxicity tests; and (3) factors affecting the adhesive properties of sulphur. Work on the chemistry phase has been published by Williams and Young (*7*), and a more extended reference will be made to it later. The present paper is a report on the second phase, toxicity, and the tests include the products made by Williams and Young.

METHODS

The tests were all made on germinating spores of *Sclerotinia cinerea*. This fungus was selected because of the sensitiveness of its spores to the toxic action of sulphur and their general adaptability to this kind of work. Germination of the spores was not greatly influenced by a hydrogen-ion concentration range between pH 2.2 and 6.0.

The spores which were used for the toxicity tests were scraped from the fruiting area of an 8-day culture and wetted with a little water in a watch glass to make a heavy spore suspension. A drop of the solution to be tested was placed on a glass slide, and spores from the heavy suspension were transferred on a dry glass rod to this drop. In each case a single transfer gave a uniform spore suspension. Equal parts of this drop were spread in a smear on each of four cover slips. These were then inverted over special cells for the germination period. The cells used were cut from a 16-mm. bore glass tubing and placed in Petri dishes in the bottom of which had been fitted a double layer of filter paper with holes cut out for placement of rings. The filter papers were wet to insure a constant and favorable moisture content. After the cover slips were laid on the rings, the Petri dishes were closed and incubated at 24° C. for 16 hours.

The Van Tieghem cell was used in some preliminary trials and was found unsatisfactory. The modified cells described above were uneven on the ends, and conditions such as high vapor pressure and collection of carbon dioxide were eliminated. The hanging-drop cultures gave uneven germination and were consequently discarded.

At the end of the germination period the cover slips were placed smear side up, and counts of the individual spores were made under low power. A spore with no germ tube or with a short dead germ tube was considered not germinated.

The acids of sulphur tested were those isolated by Williams and Young (*7*). In their work they give tests for and reactions of different compounds associated with sulphur. They include a complete list of reagents and methods of isolation of the various sulphur acids.

There have been two methods used in expressing concentration of these acids. Young and Williams (*9*) expressed the acid present in percentage. Roach and Glynne (*5*) developed for their own use a scheme in which the relative amount of sulphur designated by S is

the measure of the dilution of the acids used in toxicity tests. Neither of these expresses the concentration clearly. Different acids of the same concentration have different percentage values. There also seems to be no justification for the use of the symbol S, since there is no confirmed evidence that the atom of sulphur is toxic. Even though the different acids do dissociate into ions containing different amounts of sulphur, these ions apparently act as such, irrespective of the amount of sulphur. It does not seem advisable, therefore, to dilute a solution containing the S_5O_6 = five times to make it comparable to a solution containing the SO_4 = when used for toxicity tests. All the acids used in this work are divalent, and the expression will be given according to their hydrogen-ion concentration, e. g., 0.01 N solution contains the same number of hydrogen ions and the same number of sulphur-containing ions irrespective of the acid in solution.

TOXICITY TESTS OF ACIDS OF SULPHUR

The acids tested were sulphuric, sulphurous, dithionic, trithionic, and a mixture of tetrathionic and pentathionic. It was impossible to separate the two last mentioned, and throughout the paper these polythionic acids are called pentathionic, but the contamination with tetrathionic acid is recognized. An attempt was also made to use thiosulphuric acid, but this was found to be impossible under the conditions of the experiments because of its instability. It can not exist on the sulphur particle since it reacts immediately with sulphuric acid to form pentathionic acid. The reaction is given by Freundlich (*3*) as follows: $H_2SO_4 + 5H_2S_2O_3 \rightarrow 2H_2S_5O_6 + 3H_2O$. Sulphuric acid is normally associated with sulphur, and this precludes the existence of thiosulphuric acid. Roach and Glynne (*5*) state that their solutions containing thiosulphuric acid up to dilutions of 0.001 N and 0.0005 N were turbid. This would indicate that colloidal sulphur and relatively large quantities of pentathionic acid were present. When such solutions were tested in this laboratory they gave positive tests for pentathionic acid. Inasmuch as their solution, believed to contain thisosulphuric acid, was diluted only twice (according to their scheme of dilution), and their known solution of pentathionic acid was diluted five times, it is only natural that the first solution was more toxic, even though the toxicity of both was due to pentathionic acid.

Thirty different tests were made for each acid. These were made at the same time in double duplicate series. In solutions unaltered, except for dilution, the hydrogen ion is an important factor in its effect upon germination. This effect was eliminated in a second series by the addition of dilute sodium hydroxide. To avoid a possible change in the reaction due to spore germination, a third series was buffered at pH 5.4 with K_2HPO_4-citric-acid solution. In an effort to throw some light on the action of the S_5O_6 in neutral solutions, the acids in the fourth and fifth series were neutralized, and a buffer was added to the fifth series.

The results of toxicity tests are shown in Figures 1 to 5.

Dilute solutions, such as were used in this work, might be expected to be completely ionized. Consequently all these acids of the same normally have the same number of anions and cations, and the only variable factor is the composition of the anion. It is true that a

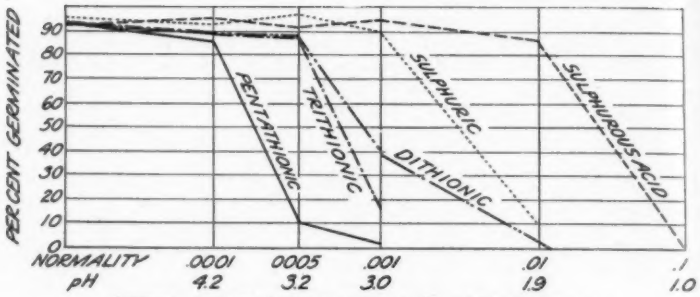

FIGURE 1.—Toxicity of acids of sulphur to spores of *Sclerotinia cinerea*

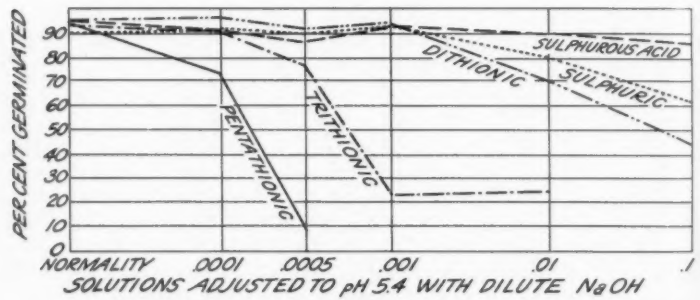

FIGURE 2.—Toxicity of acids of sulphur (altered to pH 5.4) to spores of *Sclerotinia cinerea*

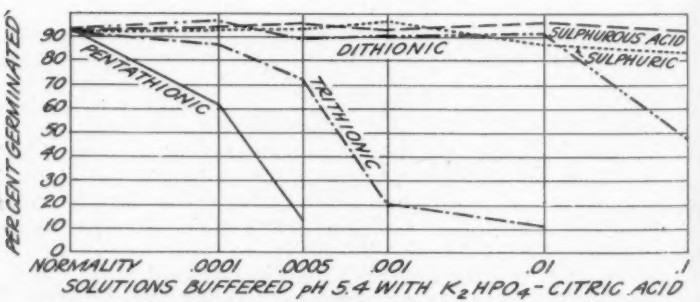

FIGURE 3.—Toxicity of acids of sulphur (buffered at pH 5.4) to spores of *Sclerotinia cinerea*

variation in the hydrogen-ion concentration will vary the percentage of germination. This is shown by the results given in Figure 1. But when this is kept constant any variation in toxicity must be attributed to the anion. The results of toxicity tests (figs. 2 and 3) show clearly the toxicity of the $S_5O_6=$, all other factors being constant or regulated. Dithionic acid is nontoxic and the trithionic acid

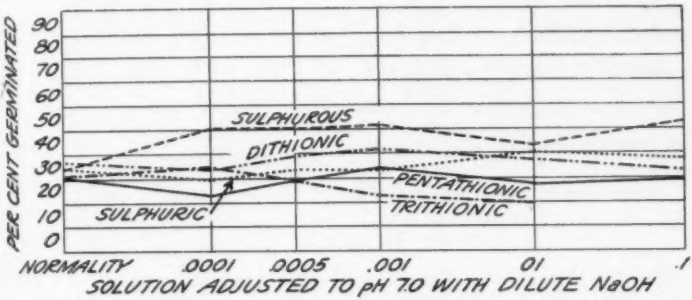

FIGURE 4.—Toxicity of acids of sulphur (altered to pH 7) to spores of *Sclerotinia cinerea*

solution only slightly so, probably owing to the pentathionic acid unavoidably present. The results given in Figures 4 and 5 show that none of the sulphur-containing ions is toxic in neutral solutions.

TOXICITY TESTS OF SULPHUR FILTRATES

After having ascertained the fact that of all the common acids of sulphur only pentathionic acid was toxic, it was thought important to

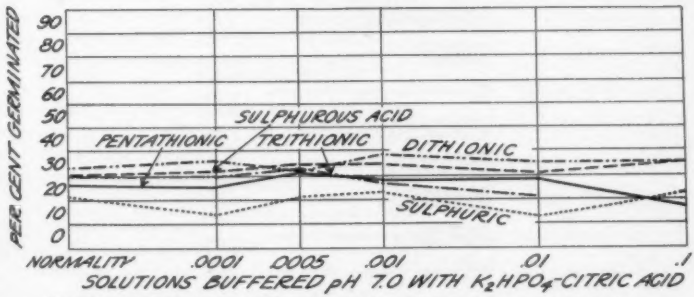

FIGURE 5.—Toxicity of acids of sulphur (buffered at pH 7) to spores of *Sclerotinia cinerea*

make a study of the acids found directly on or associated with the sulphur particle. This phase of the work was done with the filtrates of ordinary ground sulphur and flowers of sulphur. The amount of acids obtained in such filtrates depends, in a large measure, upon the extent of wetting of the sulphur. This wetting can be accomplished most easily by triturating sulphur with small amounts of water until a thick paste is formed. Ordinarily, 1 c. c. of water to 1 gm. of sulphur is sufficient. The filtrate was then obtained by using a small amount of suction.

In the analysis of this filtrate, although it was early shown that only sulphuric and pentathionic acids and traces of sulphurous acid were present, Williams and Young (7) deemed it necessary to eliminate all other acids from their suspension as well as to prove that these acids were present. Their analysis showed on the average that the acids in the filtrates from ground and flowers of sulphur were, respectively, 7.5 per cent and 12.5 per cent pentathionic acid and 92.5 per cent and 87.5 per cent sulphuric acid. Both dithionic and trithionic acids were absent. In their work 300-mesh ground sulphur and flowers of sulphur were used. Analyses of various commercial sulphur dusts were made. The total acidity of the filtrates from the different sulphur dusts varied somewhat, and the amount of pentathionic acid varied greatly. The results of the analyses are given in Table 1.

TABLE 1.—*Analysis of water filtrates from sulphur*

Sulphur used	Total acidity	Acidity minus that due to H_2SO_4 [a]	Acidity due to pentathionic acid
	Per cent	*Per cent*	*Per cent*
Flowers of sulphur	0.00222	0.000279	12.6
National sulphur	.00232	.000181	7.8
Commercial A	.00264	.000158	6.0
Commercial B	.00330	.000125	3.8

[a] Traces of sulphurous acid were present.

The toxicity of the filtrates from the same sulphurs was tested as described earlier, equal concentrations of sulphuric acid being used as checks. The results are shown in Figure 6.

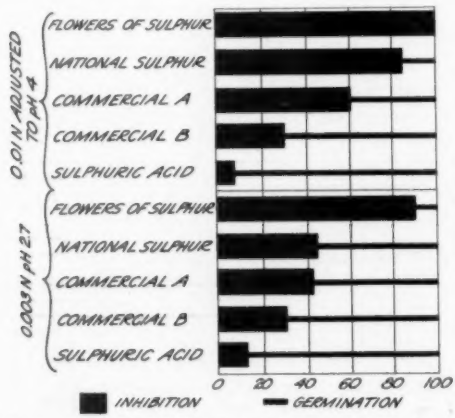

FIGURE 6.—Toxicity of filtrates of various commercial sulphur dusts, in comparison with sulphuric acid of the same concentration, to spores of *Sclerotinia cinerea*

All the filtrates which contained pentathionic acid were toxic and those with the greatest amount of pentathionic acid were the most toxic. In the checks of the same acidity, due, however, to sulphuric acid alone, germination was not inhibited.

No uncombined sulphur was present in the filtrates, and so the possibility of an increase in pentathionic acid was avoided. The Berkfeld filter was compared with extra fine filter paper in preliminary tests, and in both cases no particulate sulphur passed through with the filtrate.

A definite amount of sulphur was suspended in different amounts of water by thorough shaking. These suspensions were then used in toxicity tests. (Fig. 7.) Three hundred-mesh ground sulphur was used in all the tests unless otherwise stated. Sulphur suspensions are shown to be toxic to spores of *Sclerotinia cinerea*.

TOXICITY TESTS OF SULPHUR SUSPENSIONS AND ACID SOLUTIONS WITH THE PENTATHIONIC ACID DESTROYED

It is well known that strong acids and strong alkalis destroy pentathionic acid. A second proof of the toxicity of pentathionic acid was made possible by this characteristic reaction. When ordinary sulphur is treated with concentrated nitric acid or strong ammonium hydroxide and then washed, acid-free sulphur is obtained. It remains acid free when kept in an oxygen-free medium or in contact with one of the reagents. Pentathionic acid solutions with definite toxic properties were treated with dilute sodium hydroxide, dilute ammonium hydroxide, and the strong reagents mentioned above.

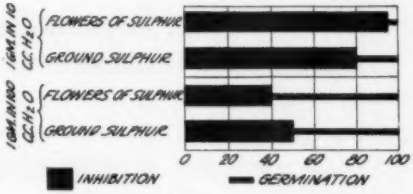

FIGURE 7.—Toxicity of sulphur suspensions (in water) to spores of *Sclerotinia cinerea*

The solutions, including checks, were adjusted to pH 5.0 with the proper reagents. These solutions were then tested for toxicity. The results are shown in Figure 8.

Either strong ammonium hydroxide or nitric acid renders a solution of pentathionic acid relatively nontoxic. The solutions tested were not freed from salts formed by the reactions of the reagents added, which in some cases, as indicated in the checks, were of such concentration that the germination was reduced by them.

Heavy suspensions of ground sulphur and flowers of sulphur were treated in the same way, and then filtrates were taken for toxicity tests. The results are summarized in Figure 9.

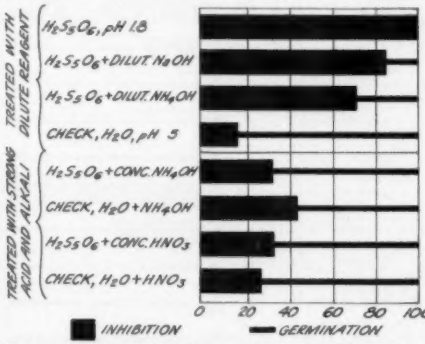

FIGURE 8.—Destruction of pentathionic acid with strong ammonium hydroxide and with nitric acid; tested by the toxicity of treated solutions, compared with untreated solutions of pentathionic acid, to spores of *Sclerotinia cinerea*

The results indicate the effect of these reagents on adsorbed pentathionic acid. Here, as in the experiment with the solutions, the toxicity was largely lost when the pentathionic acid was destroyed.

Spores in sulphur suspensions did not germinate, even though they were not in contact with the sulphur particle. It was thus evident that some of the pentathionic acid washed off the sulphur particle into the solution. Fifty grams of sulphur was washed in 100 c. c. of water by vigorous shaking in a flask, and then the wash was decanted off and saved for chemical and toxicity tests. Another 100 c. c. of water was then added, and the washing continued in this way until the wash was found to be acid free.

Most of the acid was removed in the first wash. By the third or fourth wash practically all of the acid was removed. The results of toxicity tests are shown in Figure 10.

The third and later washes from the flowers of sulphur and the fourth and later washes from ground sulphur were not toxic. A comparison with chemical tests given above shows that only those solutions containing pentathionic acid were toxic.

TOXICITY TESTS OF SULPHUR IN OXYGEN-FREE CHAMBERS

In all of the toxicity tests in the preceding experiments with acid-free sulphur only filtrates were used. Chemical tests showed that a suspension of acid-free sulphur on standing became acid due to the formation of pentathionic and sulphuric acids. Thus, acid-free sulphur could not be used in toxicity tests unless conditions were such that these acids would not be formed. This was done by testing acid-free sulphur suspensions in an oxygen-free chamber. The spore germination was tested in a suspension of acid-free sulphur in oxygen-free water in an air-tight chamber. The air was then drawn from the chamber by suction while the incoming air was passed through a pyrogallic-acid solution to remove the oxygen. The results are shown in Figure 11.

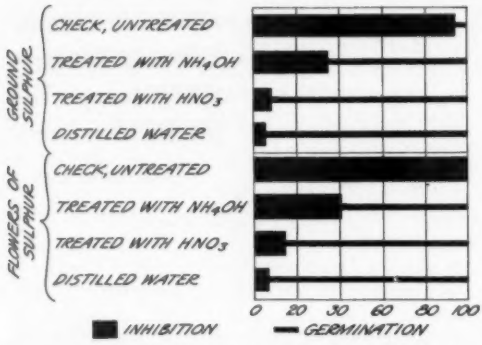

FIGURE 9.—Toxicity to spores of *Sclerotinia cinerea* of filtrates from sulphur which had been treated with strong ammonium hydroxide or with nitric acid to remove pentathionic acid

The water contained enough oxygen to permit 50 per cent of the spores to start germination. The acid-free sulphur had no apparent effect on the germination. Spores and germ tubes were not killed by contact with the sulphur particles, whereas there was no germination in a like toxicity test of ordinary sulphur. This shows that sulphur itself is not toxic but becomes so when oxidized.

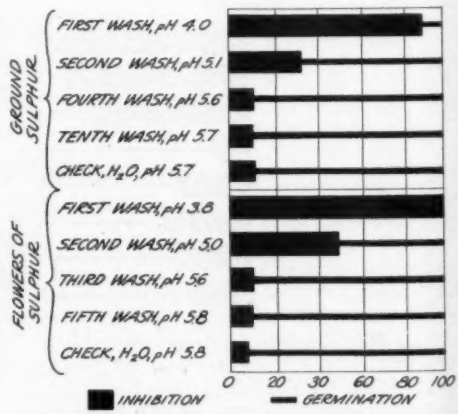

FIGURE 10.—Removal of the toxic factor from sulphur by washing with water, as expressed by the toxicity of the successive washes to spores of *Sclerotinia cinerea*

THE OXIDATION OF ACID-FREE SULPHUR

Preliminary experiments showed that a suspension of acid-free sulphur became so toxic during the 16-hour germination period that germination was stopped. In order to determine the amount of

oxygen used and the rate of oxidation, four 10-gm. samples of acid-free sulphur, prepared by the ammonium-hydroxide and the nitric-acid treatment respectively, were spread on each of two series of four large glass plates. The sulphur was then moistened and exposed to the air for a period of time.

Filtrates were taken from other samples of the same acid-free sulphur and used as checks in toxicity tests. At convenient intervals the sulphur was taken from the plates and filtrates were prepared. The filtering removed all the sulphur, and so any acidity or toxicity which the filtrate possessed would be the result of the oxidation of the sulphur during the period that the sulphur was exposed to the air. These filtrates were then used in toxicity tests. (Fig. 12.)

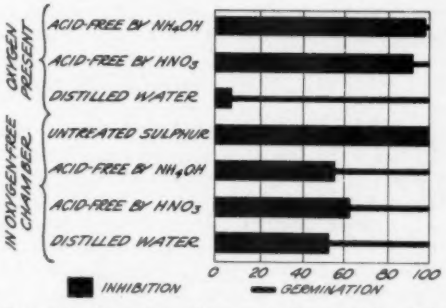

FIGURE 11.—Importance of oxygen in the production of the toxic factor in acid-free sulphur; tests made with spores of *Sclerotinia cinerea* in an oxygen-free chamber in comparison with similar tests made in the presence of oxygen

The tests show that after eight hours of aeration the sulphur had regained its normal toxicity. Even after three or four hours the sulphur was very toxic. The results of this experiment and the ones preceding it indicate that the toxic factor is an oxidation product of sulphur, and that oxygen is necessary for its occurrence.

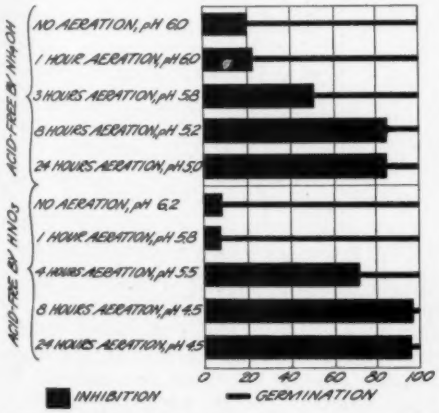
ACID-FREE BY NH_4OH
NO AERATION, pH 6.0
1 HOUR AERATION, pH 6.0
3 HOURS AERATION, pH 5.8
8 HOURS AERATION, pH 5.2
24 HOURS AERATION, pH 5.0
ACID-FREE BY HNO_3
NO AERATION, pH 6.2
1 HOUR AERATION, pH 5.8
4 HOURS AERATION, pH 5.5
8 HOURS AERATION, pH 4.5
24 HOURS AERATION, pH 4.5
0 20 40 60 80 100
INHIBITION
GERMINATION

FIGURE 12.—Effect of aeration on the production of the toxic factor in acid-free sulphur, expressed by the toxicity of filtrates of aerated sulphur to spores of *Sclerotinia cinerea*

THE EFFECT OF WEATHERING ON THE TOXICITY OF SULPHUR

It is well known that sulphur hydrolyzes rapidly at high temperatures (*3*). One of the end products of the hydrolysis and oxidation of sulphur is pentathionic acid; another is sulphuric acid. When kept at a high temperature a sulphur-lime suspension becomes less alkaline, due to the formation of insoluble and neutral salts by the reactions of sulphur acids, water, carbon dioxide, and oxygen with the calcium hydroxide. Any condition which favors these reactions causes a sulphur-lime suspension to become acid, or at least less alkaline. These conditions are present in weathering processes.

An attempt was made to expose different sulphur suspensions to conditions that would approximate weathering. Five hundred grams

of acid-free sulphur, sulphur plus a trace of lime, and sulphur-lime (85–15) mixtures were made up, and each was divided into 50-gm. samples. Each sample was put in a flask and well mixed with 50 c. c. water. This suspension was then poured into a large open dish. Duplicate samples of each suspension were exposed to sunlight, heavy shade, laboratory conditions, and at a temperature of 80° C. One sample of each suspension was kept as a check in a closed flask. The other sample was titrated to determine the initial acidity of the suspension.

Each day the water that had evaporated was replaced. At the end of four days the suspensions were placed in flasks and mixed thoroughly. Two c. c. of each suspension were removed for toxicity tests. The suspensions were then titrated with dilute acid or alkali.

The suspensions for toxicity tests were filtered, but no attempt was made to free the filtrates of soluble salts. Therefore, the germination tests were not indicative of the amount of pentathionic acid formed. The results of change in the acidity are recorded in Table 2.

TABLE 2.—*The effect of weathering on the acidity of various sulphurs and sulphur mixtures*

Sulphur	Initial acidity	Treatment	Increased acidity
Acid-free sulphur	0.00006	Sunlight	0.01052
		Heavy shade	.00305
		Laboratory	.00330
		Heat; 80° C	.01870
		Check flask	.00021
Ordinary sulphur	.00405	Sunlight	.00198
		Heavy shade	.00119
		Laboratory	.00055
		Heat; 80° C	.00544
		Check flask	.00013
Sulphur plus lime	−.00069	Sunlight	.00252
		Heavy shade	.00034
		Laboratory	.00145
		Heat; 80° C	.00026
		Check flask	.00165
Sulphur-lime; 85–15 mixture	−5.71900	Sunlight	.87700
		Heavy shade	.14850
		Laboratory	.02200
		Heat; 80° C	2.52100
		Check flask	−.00076

There was only a slight increase in acidity in the ordinary sulphur. The mixture was hardly wet at all, and because of the formation of a film practically no evaporation took place. About the same condition was present in the neutral sulphur-lime mixtures. In the acid-free sulphur and the sulphur-lime (85–15) mixtures the increase in acidity was definite in all exposed samples. They were wet to form a smooth paste. Evaporation was so great that 50 c. c. of water was added each day to all but the checks in the flasks. A conspicuous layer of white crystals formed on the surface of the sulphur-lime (85–15) mixture. These crystals were insoluble in water. By far the greatest increase in acidity occurred at the high temperature.

The results of this experiment indicate that water is necessary for the formation of acids on sulphur, and that high temperatures are favorable to the reaction.

FIELD TESTS WITH OXIDIZED SULPHUR

Since the ultimate goal of this work is to produce a dusting sulphur that has fungicidal properties equal to sprays, it seemed advisable to try some of the known sulphur-oxidizing agents. Lee and Martin (*4*) were successful in increasing the toxicity of sulphur by the use of potassium permanganate and nitric acid. In previous experiments the writers have used manganese dioxide. These three oxidizing agents were used in field tests for the control of apple scab, *Venturia inaequalis*, in connection with a number of other experimental dusts. The permanganate dusts were 1 per cent and 2½ per cent. Manganar, which employs manganese dioxide as the oxidizing agent, was used at the rate of 4 per cent for preblossom application and 10 per cent during the postblossom periods. The 300-mesh ground sulphur was used for all dusts except the commercial dusting sulphur. The dust applications were first made in accordance with the timing of the discharge of the spores of the apple scab fungus and, later, ahead of heavy rain periods. In all, seven applications were made. The experiment was conducted in two orchards, one in southern Ohio on Rome Beauty, the other in northern Ohio on Stayman Winesap. The table does not include the results from the southern orchard, since all dusts used were effective. Conditions were not favorable for scab development and infection was not severe. In northern Ohio infection was severe, especially during June and July. The results are given in Table 3.

TABLE 3.—*Results of field tests with oxidized sulphurs*

Material	Total fruit counted	Moderate scabbed	Severe scabbed	Total scabbed
	Number	*Number*	*Number*	*Per cent*
Sulphur+$KMnO_4$ (2½ per cent)	602	22	15	6.1
Sulphur+$KMnO_4$ (1 per cent)	726	61	22	11.4
Sulphur+HNO_3 (¼ per cent)	665	55	40	14.3
Sulphur+Manganar (4 per cent)	675	32	17	7.3
Pure 300-mesh sulphur	1,262	196	106	23.9
Commercial dusting sulphur	655	116	29	22.1
Check				98.5

The results show a marked increase in effectiveness in controlling apple scab. The permanganate plots showed rather severe leaf injury from both leaf drop and scald. This injury came as soon as arsenate of lead was added to the dust and was expected. It is doubtful if the two can be used together in a dust. Manganese arsenate-sulphur mixture gave some leaf injury in one test only, and this was not severe.

DISCUSSION

The results of this work confirm the main point established by Young (*8*) that the toxic factor of sulphur is pentathionic acid. It seems conclusively established that this factor is an oxidation product of sulphur. The exact manner in which it is formed is at present not definitely known, though it is reasonable to expect that the theory and reactions given by Williams and Young (*7*) are correct. They state that it is probably formed as follows:

$$S + O_2 \rightarrow SO_2$$
$$SO_2 + H_2O \rightarrow H_2SO_3$$
$$H_2SO_3 + S \rightarrow H_2S_2O_3$$
$$5H_2S_2O_3 \rightarrow 2H_2S_5O_6 + 3H_2O.$$

Sulphur dioxide is present only in very small quantities and is considered by them a transitory product in the oxidation of sulphur.

The manner in which pentathionic acid is toxic has not been definitely studied. The above results indicate plainly that the $S_5O_6^=$ is at least directly or indirectly responsible. All factors enhancing its production increase toxicity. Whether this ion breaks down in the presence of leaf tissue or germinating spores with the production of other toxic substances is as yet undetermined. The writers have been able to detect hydrogen sulphide on ordinary forms of sulphur when the latter is on leaves or in contact with spores. The theory that hydrogen sulphide is produced by the action of fungus spores on the sulphur particle and is the toxic factor seems untenable, since filtrates from sulphur suspensions contain no particulate sulphur and yet are toxic.

SUMMARY

Pentathionic acid was the only one of the acids tested which showed a marked degree of toxicity. Sulphuric, sulphurous, dithionic, and trithionic acids were only slightly, if at all, toxic.

Sulphur filtrates secured from wetted sulphur contained pentathionic acid and were toxic.

Sulphur that was freed of pentathionic acid, either by strong alkali or acid, or by washing, was not toxic.

Acid-free sulphur which was aerated eight hours regained its normal toxicity. Qualitative tests for pentathionic acid were positive.

Sulphur-lime mixtures when exposed to air, light, and heat in the presence of moisture tended to become less alkaline. Closed checks in the laboratory did not change in reaction.

Field tests confirmed the laboratory results, inasmuch as sulphur, to which an oxidizing agent was added, was more effective than ordinary sulphur in the control of the apple-scab disease.

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